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(54) Title: XYLOSYLTRANSFERASE AND ISOFORMS THEREOF

(57) Abstract: The invention relates to the isolation, purification and characterization of the enzyme xylosyltransferase (defined as "XT"). The invention describes for the first time that XT occurs in at least two isoforms ("XT-I", "XT-II"). The invention relates furthermore to the recombinant cloning and expression of human and rat XT-I and XT-II and discloses their DNA and protein sequences. The enzymes according to the invention can be used as therapeutic agents and as diagnostic markers, e.g. for the determination of enhanced proteoglycan biosynthesis, and as biochemical markers for determination of several pathological processes such as systemic sclerosis.

Xylosyltransferase and Isoforms thereof

The invention relates to the isolation, purification and characterization of the enzyme xylosyltransferase (defined as "XT"). The invention describes for the first time that XT occurs in at least two isoforms ("XT-I", "XT-II"). The invention relates furthermore to the recombinant cloning and expression of human and rat XT-I and XT-II and discloses their DNA and protein sequences. The enzymes according to the invention can be used as therapeutic agents and as diagnostic markers, e.g. for the determination of enhanced proteoglycyn biosynthesis, and as biochemical markers for determination of several pathological processes such as systemic sclerosis.

Background of the Invention

Proteoglycans are polyanionic molecules widely expressed in animal cells and virtually every tissue. These abundant molecules are present in the extracellular matrix and on the cell surface and serve a wide range of functions. They are increasingly implicated as important regulators in many biological processes, such as extracellular matrix deposition, cell membrane signal transfer, morphogenesis, cell migration, normal and tumor cell growth and viral infection (Ruoslahti, 1989, *J. Biol. Chem.* **264,** 13369-1372; Herold *et al.*, 1994, *J. Gen. Virol.* **75,** 1211-1222). Proteoglycans mediate diverse cellular processes through interaction with a variety of protein ligands. In most of these bindings electrostatic interactions with the glycosaminoglycan chains attached to the core protein are involved (Kjellen & Lindahl, 1991, *Annu. Rev. Biochem. 60, 443-475*). Thus, the biological activity of proteoglycans is intimately related to the glycosaminoglycan biosynthesis.

The sulfated glycosaminoglycans chondroitin sulfate, heparan sulfate, heparin and dermatan sulfate are bound to the proteoglycan core protein by a xylose-galactose-galactose binding region (Kjellen & Lindahl, *1991, l.c.*). UDP-D-xylose:proteoglycan core protein β-D-

various species (*Hoffmann et al., 1984, Connect. Tissue. Res. 12, 151-164*), and it was shown that the enzyme is secreted from the endoplasmatic reticulum into the extracellular space together with chondroitin sulfate proteoglycans (Kähnert et al., 1991, *Eur. J. Clin. Chem. Clin. Biochem. 29, 624-625;* Götting et al., 1999, *J. Invest. Dermatol. 112, 919-924*). However, the processes resulting in the release of XT from the endoplasmatic reticulum or the Golgi compartments and the role of XT in the extracellular matrix are not yet known .

As XT is the initial step enzyme in the biosynthesis of the glycosaminoglycan linkage region and as it is secreted into the extracellular matrix to a great extent, XT activity was proposed to be a diagnostic marker for the determination of an enhanced proteoglycan biosynthesis and of tissue destruction (Weilke et al., 1997, *Clin. Chem. 43, 45-51*). XT activities in the synovial fluid were found to be significantly increased in chronic inflammatory joint diseases (Kleesiek et al., 1987, *J. Clin. Chem. Clin. Biochem. 25, 473-481*). Recent studies have shown that serum XT activity is a biochemical marker for the determination of fibrotic activity in systemic sclerosis (Götting et al.,1999, *l.c.;* Götting et al., 2000, *Acta Derm. Venereol. 80, 60-61.*).

Up to now there was no success to isolate or characterize xylosyltransferase (XT), however some methods were described to measure and determine the activity of said enzyme from blood or body fluid samples of patients showing pathological effects such as scleroderma or chronic joint diseases (Stoolmiller, 1972, *J. Biol. Chem. 247, 3525-3532).* The samples were incubated with UDP-[^{14}C]xylose and an appropiate acceptor. The incorporated radioactivity indicated the amount of XT activity. Acceptors used so far are proteoglycans, silk fibroin (Campbell et al., 1984, *Anal. Biochem. 137, 505-516)* and several peptides (Bourdon et al., 1987, *Proc. Natl. Acad. Sci. USA 84, 3194-3198).* However, the all activity tests used herein did not allow precise determination of the lower XT activity in serum. A more specific acceptor protein is recombinant bikunin, the inhibitory component of human inter-α-trypsin inhibitor. Bikunin carries a single chondroitin, which is essential for the structure of the inhibitor. The chondroitin sulfate attachement site in the N-terminal region contains all elements responsible for recognition by XT. The complete recognition sequence is composed of the amino acids a-a-a-a-G-S-G-a-b-a, with a = E or D and b = G, E or D. This sequence was confirmed by determination of the Michaelis-Menten (K_m) constants for *in vitro* xylosylation of different synthetic proteins and peptides using an enriched XT preparation from conditioned cell culture

supernatant of human chondrocytes. The constant was determined to be 22 μM, which was decreased 9-fold in comparison to deglycosylated core protein from bovine cartilage (188 μM) (Brinkmann et al., 1997, *J. Biol. Chem. 272, 11171-11175)*. With recombinant bikunin as acceptor, a sensitive assay was developed that allows precise determination of XT activity in human serum and other body fluids *(see:* Weilke et al., *1997, l.c.)*. Using this assay an increased xylosyltransferase activity was determined in blood of patients with sclerotic processes of scleroderma, closely related to an elevated proteoglycan biosynthesis (Götting et al., 1999, l.c.*)*.

The biosynthesis of glycosaminoglycans requires the coordinated action of a large number of glycosyltransferases. Isolation and cloning of these glycosyltransferases has been targeted for a long time, since the majority of these enzymes are only present in minute amounts. The structure and sequence of the glycosyltransferases involved in biosynthesis of the common glycosaminoglycan-protein linkage region has long remained unknown. Recent cDNA cloning of galactosyltransferase I (Okajima et al., 1999, *J. Biol. Chem. 274, 22915-22918*) and glucuruonyltransferase I (Kitagawa et al., 1998, *J. Biol. Chem. 273, 6615-6618*) identified 2 of the at least 4 enzymes involved in synthesis of the GlcAβ1-3Galβ1-3Galβ1-4Xylβ1-O-Ser structure.

Isolation, purification and characterization of XT involved in biosynthesis of the common carbohydrate-protein linkage structure has been hampered by difficulties in obtaining a sufficient amount of the source material. Since, as above mentioned, XT can be used as an additional biochemical marker for the determination of sclerotic activity in systemic sclerosis and some inflammatory disorders, it is a real need for an isolated, highly purified XT which can be produced by recombinant methods, which is necessary for diagnostic and therapeutic purposes. Moreover, the knowledge of the cDNA sequence of XT allows to use it on gene level such as in gene diagnostic or gene therapy.

Definitions

- Above and below the term "XT" means xylosyltransferase (UDP-D-xylose:proteoglycan core protein β-D-xylosyltransferase (EC 2.4.2.26) deriving from any origin and includes all possible isoforms, if not otherwise pointed out. The term "hXT" has the meaning of human XT; the term "rXT" of rat XT. The terms "XT-I" , "XT-II", "hXT-I", "hXT-II", "rXT-I2,

"rXT-II" mean the specific isoforms 1 and 2 of XT according to the invention, wherein h and r have the indicated meanings.

- Above and below the term "protein" means a protein, a protein fragment or a peptide, if not otherwise explained.
- Above and below the term "recombinant protein" is defined as a protein which was produced by recombinant and biotechnological methods.
- Above and below the term "XT" or "XT protein(s)" has the meaning of (a) protein(s) deriving from any source, if not otherwise stated out, having the biological activity and / or function *of UDP-D-xylose:proteoglycan core protein ß-D-xylosyltransferase.*
- Above and below the term "a protein having the biological activity of UDP-D-xylose:proteoglycan core protein ß-D-xylosyltransferase " is defined as a protein which has XT – identical or XT – like functions and activities and comprises XT itself and possible mutations, variants, isoforms thereof, including insertions, deletions and substitutions of one ore more amino acids. The term includes also fragments or longer forms of XT as well as dimeric or multimeric forms thereof showing XT functions.
- Above and below the term "isoform" means a second naturally occurring enzyme having the same biological activity as a first naturally occurring enzyme, however differing by another amino acid sequence.

Summary of the Invention

It was found that XT can be purified 4,700-fold with 1% yield from serum-free JAR choriocarcinoma cell culture supernatant. The isolation procedure includes a combination of ammonium sulfate precipitation, heparin affinity chromatography, ion exchange chromatography and protamine affinity chromatography. Amongst other proteins an unknown protein was detected by matrix-assisted laser desorption ionization mass spectrometry-time of flight analysis (MALDI-TOF) in the purified sample. The molecular weight of this isolated and purified XT protein was determined as 120.000 by SDS-polyacrylamide gel electrophoresis. The isolated protein was enzymatically cleaved by trypsin and endoproteinase Lys-C. Peptide fragments were sequenced by Edman degradation. Searches with the amino acid sequences in protein and EST databases showed no homology to known sequences. XT was enriched by immunoaffinity chromatography with an immobilized antibody against a synthetic peptide deduced from the sequenced peptide fragments and was specifically eluted with the antigen. In

addition, XT was purified alternatively with an aprotinin affinity chromatography and was detected by western blot analysis in the enzyme-containing fraction.

Based on the partial amino acid sequence of their isolated and purified new enzyme (XT) derived from human JAR choriocarcinoma cell culture supernatant a novel cDNA was isolated according to the invention, encoding human XT-I enzyme using the degenerate reverse transcriptase-polymerase chain reaction method. The enzyme belongs to a novel family of glycosyltransferases having no homology to proteins of prior art. 5'- and 3'-RACE were performed to isolate a novel cDNA fragment of 3726 bp with a single open reading frame encoding at least 827 amino acids with a molecular mass of 91.000. The human XT-I gene was located on chromosome 16p13.1 using radiation hybrid mapping, and extracts from CHO-K1 cells transfected with the XT-I cDNA in an expression vector exhibited marked XT activity. Furthermore, a new 3608-bp cDNA fragment encoding a novel protein of 865 amino acids was also isolated by PCR using degenerate primers based on the amino acid sequence of human XT-I. The amino acid sequence of this XT-II isoform displays 55% identity to the human XT-I. The XT-II gene is located on chromosome 17q21.3-17q22, and the exon/intron structure of the 15 kb gene was determined. RT-PCR analyses of XT-I and XT-II mRNA from various tissues confirmed that both XT-I and XT-II transcripts are ubiquitously expressed in the human tissues, although with different levels of transcription. Furthermore, the cDNAs encoding XT-I and XT-II from rat were cloned. The deduced amino acid sequences of rat xylosyltransferases displayed 94% identity to the corresponding human enzyme.

Therefore, it is an object of this invention to provide the following subject-matters:

- An isoform of *UDP-D-xylose:proteoglycan core protein ß-D-xylosyltransferase*(XT);
- a protein comprising a sequence of said isoform or a fragment thereof, having the biological activity of XT;
- a corresponding protein deriving from human or rat sources (hXT, rXT);
- a corresponding protein isolated from specific human tissue, wherein said hXT has a molecular weight of 120.000 under SDS PAGE conditions;
- a corresponding recombinant protein, wherein said protein is hXT-I comprising at least 827 amino acids and having the amino acid sequence as depicted in Fig. 7B;

- a corresponding isoform of hXT-I, termed as hXT-II, comprising 865 amino acids, exhibiting approximately 55% overall sequence identity to human hXT-I, and having, in more detail, the amino acid sequence as depicted in Fig. 8B.
- a process for isolating and purifying a protein having the biological activity of human UDP-D-xylose:proteoglycan core protein ß-D-xylosyltransferase (EC 2.4.2.26), said process is characterized by the following steps:
  - (i) culturing from human tissue showing an enhanced XT activity, preferably from JAR choriocarcinoma cells (ATCC HTB-144), and harvesting the cell culture supernatant,
  - (ii) fractionated ammonium sulfate precipitation of the supernatant of step (i),
  - (iii) heparin affinity chromatography of the precipitate of step (ii),
  - (iv) ion exchange chromatography of the step (iii) product, and
  - (v) affinity chromatography of the step (iv) product, and optionally
  - (vi a SDS-Polyacrylamide gel elektrophoresis of step (v);
- recombinant forms of hXT and rXT, the corresponding DNA sequences (Fig. 7A, 8A, 9, 10) included as well as suitable expression vectors and expression host cell systems;
- antibodies directed against any of the above or below mentioned XT proteins and their uses in immunological assays and diagnostic tools for determining said XT proteins;
- pharmaceutical compositions comprising a XT protein as defined above and below, optionally together with a suitable pharmacologically acceptable carrier, diluent or excipient;
- uses of said XT proteins for the manufacture of a medicament for the treatment of XT relevant diseases and disorders, wherein the xylosyltransferase enzymes according to the invention can either be used directly as therapeutic drug in pathological situations where a deficiency of said enzyme and its isoforms can be detected;
- uses of said XT enzymes for the manufacture of a medicament for the treatment of diseases and disorders which are caused or accompanied by increased levels of said enzymes, e.g. in sclerotic diseases and chronic joint diseases, wherein said medicament is an inhibitor or antagonist of said XT proteins;
- uses of said XT proteins as diagnostic markers for the diagnosis of above- and below-mentioned diseases and pathological symptoms and uses of said DNA molecules as gene markers;

- methods of screening for compounds which are capable to inhibit the activity of said XT proteins according to the invention using known methods for determining the activity of XT.

Detailed Description:

General

UDP-D-xylose: proteoglycan core protein ß-D-xylosyltransferase can be isolated and purified from JAR choriocarcinoma cell culture. The isolated protein according to the invention is a single stranded polypeptide with a molecular weight of 120.000. The protein was enzymatically cleaved and eleven peptide fragments were sequenced by Edman degradation (see Examples). XT is present only in very small amounts in animal tissues but unlike other glycosyltransferases, more than 90% of XT is enriched in the medium of cultured cells. The highest secretion of XT activity was measured in JAR choriocarcinoma cell culture, in which sternal cartilage chondrocytes and 21 different human cell lines were examined. To produce a highly enriched XT solution for the isolation of XT, JAR choriocarcinoma cells were adapted to hollow fiber culture conditions using a novel bioreactor (TECNOMOUSE) and Ultradoma-PF medium without serum addition as nutrient. For purification of XT a combination of classic separation methods and new affinity matrices was employed. Therefore, a heparin matrix was used as an affinity ligand for the XT. When applied to immobilized heparin, XT was completely adsorbed at the matrix and the XT activity was eluted only with a high salt concentration after most contaminating proteins have been removed from the matrix. Protamine chloride is well-known as cationic activator for several sulfotransferases, so the effect of protamine chloride on the XT was investigated. An increased XT activity was measured when protamine chloride was added to the XT assay solution (see Examples), indicating an interaction of these arginine rich proteins with XT. Therefore, a protamine chloride affinity matrix was synthesized using an aldehyde activated perfusion medium as support. The interaction of XT with this affinity matrix resulted in a 13.6 times enrichment of XT. The protamine chloride affinity chromatography was the most efficient purification step during the isolation of the XT. Immobilized aprotinin, a Kunitz-type proteinase inhibitor, was found to be another appropriate affinity matrix for enrichment of XT, as it was able to adsorb XT quantitatively. Therefore, it was used for alternative purification of XT. Different lines of evidence showed that the isolated protein corresponds to the XT: (a) XT activity was enriched

using immobilized antibodies raised against a synthetic peptide deduced from the 120.000 protein, and the XT activity could be competitively eluted with this peptide. (b) Immunoblot analysis of aprotinin affinity purified XT corresponds with the 120.000 protein. However, non-reducing and non-denaturating gel filtration chromatography with heparin affinity-purified XT from JAR cell culture supernatant shows an additional peak of XT activity at a molecular weight of approximately 500.000. XT is associated with proteoglycans. Treatment of XT with N-glycosidase F resulted in a decrease of the molecular weight from 120.000 to 116.000, suggesting that the XT is a glycoprotein. A comparison of the molecular mass of XT with other glycosyltransferases involved in biosynthesis of proteoglycans shows that the XT is larger than the other enzymes. Another difference is that nearly all proteoglycan glycosyltransferases are tightly bound to the membrane of the endoplasmic reticulum, whereas XT is secreted into the extracellular space. XT according to the invention contains like many glycosyltransferases a DxD motif, suggesting that this motif is involved in binding the metal-ion cofactor and the donator substrate (Gastinel et al., 1999, *EMBO J.* 18, 3546-3557). A DxD sequence was also found in peptide 8 obtained from the enzymatically cleaved XT.

The invention discloses for the first time the molecular cloning and expression of human and rat XT. Surprisingly, XT is found in at least two isomeric forms, which are termed according to the invention as XT-I and XT-II. Based on the amino acid sequence a novel cDNA was cloned, which encodes a protein of at least 827 amino acids with a molecular mass of 91.000. A DxD motif was identified in the XT-I amino acid sequence using hydrophobic cluster analysis. This motif has been observed in many glycosyltransferases, and is involved in the coordination of a divalent cation in the binding of the nucleotide-sugar (Breton & Imberty, 1999, *Curr. Opin. Struct. Biol.* 9, 563-571.). The translation initiation codon of the XT-I cDNA could not be yet cloned probably due to strong secondary structures in the 5'-region of the XT-I mRNA. The XT-I protein isolated from human JAR choriocarcinoma cell culture supernatant migrated on SDS-PAGE with a molecular mass of 120.000 and the protein size could be further reduced after N-glycanase digestion. These findings indicate that the cloned cDNA represents at least 90% of the coding region of human XT-I.

Another cDNA was completely identified from human placenta RNA, which was similar but not identical to the hXT-I cDNA. The new cDNA encodes a protein of 865 amino acids with a molecular weight of 97.000. This novel protein termed XT-I has a proline-rich region located

near the amino-terminus and the type II transmembrane topology characteristic of many other glycosyltransferases cloned to date. The hXT-II protein was similar to the human XT-I with an overall sequence identity of 55%. The similarity of the predicted amino acid sequences was low (<10%) at the amino-terminal end. However, large stretches at the C-terminal region, where the catalytic domain was found to be located in glycosyltransferases, are very conserved with an identity of more than 80% in both proteins. These findings let conclude that the XT-II gene encodes another human xylosyltransferase, although the catalytic activity and the biological role of XT-II remain to be elucidated in detail.

Since alterations in XT activity have been reported to be associated with fibrotic and sclerotic alterations of connective tissue (Götting *et al.*, 1999; Götting *et al.*, 2000, l.c.), the present findings provide molecular tools to study the function and the regulated expression of human XT as well as the molecular mechanisms of these diseases.

Production of XT

JAR choriocarcinoma cells which had been adapted to growth in the serum-free Ultradoma-PF medium secreted XT activity into the cell culture supernatant. During the exponential growth XT activity and total protein concentration in the supernatant of a traditional cell culture system (T-flasks) were determined as 0.2 mU/l and 0.1 g/l, respectively. The cells were cultivated using three "Tecnomouse"® bioreactor systems. Each bioreactor contained five culture casettes. About 10^7 cells per culture casette were inoculated and medium probes of about 0.5 ml were taken every day to determine the viability of the cells and the glucose, and lactate concentration as well as the XT activity of the cell culture supernatant. The cells amounted in the probes varies from 10^5 to 10^7 cells per ml with viability between 40 and 70%. XT production increased within three weeks after cell inoculation, reaching a plateau of approximately 30 mU/l. After three months the culture cassette was removed. Harvesting was carried out every two days, collecting 10 ml cell culture supernatant per culture cassette. The cell-free supernatant was collected and stored at -75°C. Mean XT activity in the supernatant of the high density culture was determined as 29.0 mU/l, while the total protein concentration was estimated as 4.8 g/l. In total 18.5 l high density cell culture supernatant was collected and yielded 535.8 mU XT.

Isolation and purification of XT from cell culture supernatant

XT was purified form 18.5 l supernatant (equivalent to 2.000 l normal cell culture supernatant) of serum-free cultivated JAR choriocarcinoma cells to an apparent homogeneity of about 4700-fold purification. A summary of the purification steps for isolation of XT is shown in Table 1. XT activity of the crude supernatant was approximately 0.006 mU/mg protein. The purification method according to the invention comprises four, preferably five different steps. It is possible, that one step can be achieved more than once if necessary.

Step 1: Fractionated ammonium sulfate precipitation XT of the ammonium sulfate precipitable fraction was dissolved in 0.46 l buffer A with solubilization of 79.5% of the original activity.

Step 2: Heparin affinity chromatography on POROS 20 HE 4 ml of XT-enriched solution from step 1 was loaded onto the POROS 20 HE column. XT activity was completely retained on the column. More than 70% of total protein passed through the column. Contaminating protein was eluted at a low NaCl concentration. 44% of the XT activity bound to the heparin matrix emerged at 0.5 M NaCl (Fig. 1A).

Step 3: Ion exchange chromatography on POROS 20 HQ 4 ml of the desalted XT-containing fraction from step 2 was loaded onto the POROS 20 HQ column equilibrated in buffer A. More than 98% of the XT activity bound to the resin. The column was then eluted stepwise with NaCl in buffer A (Fig. 1B). XT-containing fractions were collected.

Step 4: Affinity chromatography on protamine chloride The product of step 3 was desalted and concentrated using dia- and ultrafiltration. 100 µl of the protein solution was applied to the POROS protamine chloride column previously equilibrated with buffer A. Approximately 95% of the transferase activity bound to the column, whereas 75% of the contaminating protein did not. Additional proteins were eluted with buffer A containing low NaCl concentrations. Enzyme activity was eluted at approximately 0.15 M NaCl (Fig. 1C). The enzyme activity was stable for at least 6 months at -75°C.

Step 5: SDS-Polyacrylamide gel electrophoresis (SDS-PAGE) XT-containing fractions from step 1-4 were subjected to SDS-PAGE on a 4-12% gradient polyacrylamide gel (Fig. 2, panel A). Coomassie-stained protein bands were excised and characterized by MALDI-TOF mass spectrometry after tryptic digestion. The molecular weight of an unknown protein was determined as 120.000 (Fig. 2, panel B).

Step 5 is an optionally step.

Table 1:

Summary of single purification steps employed for isolation of XT from 18.5 l of high density JAR cell culture supernatant.

Step	Volume	Total activity	Total protein	Spec. activity	Purification	Recovery
	ml	10-3 x units	mg	10-3 x units/mg	-fold	%
JAR high density cell culture Supernatant	18,500	535.8	89,355.0	0.006	1	100
Ammonium sulfate precipitation	460	426.0	8,937.8	0.048	8	79
Heparin affinity chromatography	50	108.3	473.0	0.229	40	35
Ion exchange Chromatography	5	91.0	43.1	2.090	348	17
Protamine affinity chromatography	1	6.83	0.24	28.458	4,743	1

Amino acid sequence analysis of XT. The MW 120.000 protein from the excised band was digested with trypsin and endoproteinase Lys-C. The proteolytic fragments were separated by reversed-phase HPLC, and selected peptides were subjected to automatic amino acid sequence analysis. Table II shows the obtained 11 amino acid sequences determined by Edman degradation and mass spectrometry.

Table II. X represent a not identified residue. The masses of three peptides were observed in the MALDI mass spectrum of the enzymatically digested MW 120.000 protein. Calculated mass values were obtained from the sequence obtained.

Peptide	observed mass ($M+H^+$)	calculated mass ($M+H^+$)
(1) ELGAK		
(2) ELLK		
(3) DMNFLK		
(4) IASPPSDFGR	1045.5	1045.5
(5) LLLD		
(6) DFENVDNSNFAPR	1524.7	1525.7
(7) PTFFAR		
(8) LQFSEVGTDXDA		
(9) ELGAVKPDGRL	1152.6	1153.7
(10) ELLKRKLEQQEK		
(11) LGLLMPEK		

Immunochemical detection of XT

Polyclonal antibodies against the synthetic peptide CSRQKELLKRKLEQQEK deduced from the peptides 2 and 10 of the enzymatically cleaved MW 120.000 protein were covalently bound on POROS 20 PA. About 50% of the XT activity of an applied sample was bound (Fig. 3, panel A) when a partially purified XT sample obtained by heparin affinity chromatography (purification step 2) was loaded onto the column. 58% of the adsorbed XT activity was eluted with 150 mM NaCl, and the rest was eluted with 12 mM HCl. Furthermore, the adsorbed XT activity was also eluted from the solid phase when 100 µl (1 mg/ml) of the synthetic peptide was added to the mobile phase (Fig. 3, panel C). When immobilized preimmune serum was used as affinity matrix (negative control) no XT activity was adsorbed to or eluted from the matrix (Fig. 3, panel B). The desalted XT fraction after heparin affinity chromatography (purification step 2) was loaded on an aprotinin affinity column. The elution profile shows four major protein peaks (Fig. 4, panel I). 61% of the XT activity adsorbed to the aprotinin matrix emerged at 0.30 M NaCl and another 21% at 0.54 M NaCl. A single MW 120.000 band of the XT-containing fractions was detected by western blot analysis with the polyclonal antibodies (Fig. 4, panel B).

Determination of the molecular weight of XT

100 µl of heparin affinity purified XT was separated under non-reducing and non-denaturating conditions using a TSK G3000 SW column. Two XT activity maxima were detected at MW 500.000 and 120.000 (Fig. 5, panel A). The molecular mass of the MW 120.000 protein was reduced about 3 % after N-glycosidase F digestion as shown by SDS-PAGE (Fig. 5, panel B).

PCR-based cloning of human XT-I cDNA

Based upon the amino acid sequence of 4 peptides degenerate primers were designed for cloning the XT-I cDNA (Figure 6). When primers SPPS1 and Lysc-inv were used in a PCR with the first strand cDNA of SW1353 chondrosarcoma poly(A)$^+$ mRNA as template, a major band of approximately 690 bp was observed. After subcloning and sequencing a previously unknown DNA sequence was obtained from clone pCG114-29. PCR amplification with the primers DF1 and a mixture of Inv2b and Inv2c resulted in a 1724 bp fragment, which was subcloned and sequenced. The deduced amino acid sequence of the clone pCG111-4 was identical to 6 of the sequenced peptides from human XT-I. The cloning strategy of rapid

amplification of cDNA ends (RACE) (*Chenchik et al., 1996, BioTechniques 21, 526-534*) was employed to clone the complete coding sequence of the XT-I cDNA. The largest DNA fragment obtained from the 3'-RACE reaction was 1.6 kb and consisted of a 3'-untranslated region of 1240 bp. The 5' RACE reaction was performed with different primers derived from the nucleotide sequence of XT-I cDNA, but the 5'-untranslated region of human XT-I cDNA could not be cloned using this method. All DNA fragments obtained contained just the known cDNA sequence and an additional 80 bp of coding sequence. Thus, a PCR-based screening approach using cDNA libraries as template was employed for cloning of the 5'-untranslated region. All DNA fragments obtained from the screening of 3 different human cDNA libraries stopped at the same nucleotide, indicating that stable secondary structures of the XT-I mRNA prevent the synthesis of cDNA of the 5' untranslated region during the reverse transcription reaction. However, the translation initiation codon of human XT-I could not be yet cloned. The combined cDNA of human XT-I contained 3726 bp (Fig. 7A) with a single open reading frame encoding at least 827 amino acids with a molecular mass of a least 91.000. The deduced amino acid sequence contained 3 potential N-glycosylation sites (Figure 7B). Analysis of the amino acid sequence using the hydrophobic cluster analysis (Gaboriaud *et al.*, 1987,) revealed the presence of a common DxD motif at position 182, which has been shown to be essential for binding nucleotide-sugars in glycosyltransferases .

PCR-based cloning of human XT-II isoform cDNA

The degenerate primers PFF-sense and Lysc-inv1 which were designed upon the amino acid sequence of proteolytic cleaved peptides of human XT-I were used in a PCR reaction with first strand cDNA of placenta poly(A)$^+$ mRNA as template. The PCR amplification resulted in a minor band of 1.1 kb, which was cloned and sequenced. The determined nucleotide sequence was similar but not identical to the XT-I cDNA sequence, indicating that the fragment encodes a XT-II isoform. To clone the complete coding sequence of the novel cDNA, the RACE strategy was employed. The cDNA finally obtained was 3608 bp (Figure 8A) and contained a single open reading frame encoding a protein of 865 amino acids with a molecular mass of 97.000. The 3'-untranslated region is 850 bp and a 5'-untranslated region of 149 bp was identified with an in-frame stop codon upstream of the ATG codon. A Kyte-Doolittle hydropathy analysis (Kyte & Doolittle, 1982, *J. Mol. Biol.* **157**, 105-132) of the deduced amino acid sequence revealed one potential membrane-spanning region consisting of 16 hydrophobic amino acid residues at position 16 to 32, which appears to result in a type II transmembrane

orientation characteristic of many of the glycosyltransferases. The predicted amino acid sequence contains a proline-rich profile pattern from position 110 to 118 and 3 potential N-glycosylation sites (Figure 8B). The human XT-II isoform protein exhibits 55% overall sequence identity to the human XT-I including stretches in the XT-II protein with more than 80% homology to the human XT-I.

Cloning of rat XT-I and XT-II cDNA

For amplification of the XT-I and XT-II cDNA from rat tissue gene-specific primers were designed based upon the nucleotide sequence of human XT-I and XT-II cDNA. Theses oligonucleotides were employed in PCR, 5' RACE and 3' RACE reactions with rat liver cDNA as template and a 2593 bp cDNA fragment (Fig. 9) coding for rat XT-I was obtained. 5' RACE reactions using a diversity of gene-specific primers were performed for identification of the 5' end of the XT-I cDNA. All cDNA fragments obtained stopped at exactly the same nucleotide position as observed in the human XT-I cDNA, indicating that stable secondary structures of the XT-I mRNA inhibit cDNA synthesis of the 5' region. The XT-II cDNA fragment finally obtained after PCR amplification with rat liver cDNA was 2782 bp (Fig. 10) and included the entire coding region of rat XT-II. The deduced amino acid sequences of XT-I and XT-II (SEQ IDs 6, 8) from rat each displayed 94% identity to the corresponding human xylosyltransferase. Alignment of all amino acid sequences revealed the presence of highly conserved amino acid clusters in the central and carboxyterminal regions of the proteins.

Identification of the chromosomal localization of the human XT-I and XT-II genes

The gene coding for human XT-I could be assigned to the short arm of chromosome 16 at the region 16p13.1. The radiation hybrid mapping using the Genebridge 4 radiation hybrid screening panel located the position of the PCR fragment 5.98 cR distal to the STS marker CHLC.GATA42E11 and 4.40 cR proximal to marker D16S499. The XT-II gene was identified on chromosome 17 at the position 17q21.3-17q22 and was found to be located distal to the STS marker WI-11424 and proximal to the marker WI-14315.

Expression of recombinant XT

To prove the function of the cDNA product, a recombinant form of XT-I was generated by fusing the cloned XT-I to an aminoterminal peptide tag. The fused protein was expressed in

CHO-K1 cells and absorbed from the medium by immunoprecipitation with anti-*Xpress*-antibodies and protein G agarose beads to eliminate endogenous XT activity. The enzyme-bound beads were used as an enzyme source and assayed for XT activity as shown in Table 3 (see below). No detectable XT activity was recovered by the affinity purification from a control transfection sample. The substrate specificity of the recombinant XT-I was similar to that of the XT-I isolated from human body fluids and cell culture supernatants. The recovered enzyme activity of the recombinantly expressed XT-I could be completely inhibited by addition of 250 U of heparin. As specific inhibition of human XT activity by heparin has been demonstrated previously (Kleesiek *et al.*, 1987, l.c.), these results clearly indicate that the expressed protein is the human XT. However, no enzymatic transfer of xylose to the acceptor peptides used in this study was observed when expressing XT-II fused to the aminoterminal peptide tag in CHO-K1 cells.

To identify the XT-I reaction products, the bikunin peptide was labeled with [^{14}C]-D-xylose using the XT-I-bound beads as an enzyme source. The products were subsequently subjected to the linkage-specific digestion of the bound [^{14}C]-D-xylose with α- and β-xylosidase and alkaline β-elimination. Incubation of the reaction products with β-xylosidase resulted in the release of 74% of the incorporated [^{14}C]xylose, whereas only less than 4% of the peptide-bound xylose was digested after treatment with α-xylosidase. The alkaline cleavage of the O-glycosidic linkage between the xylose and the β-hydroxyamino acid serine in the presence of borohydride lead to the liberation of more than 97% of the enzymatically transferred [^{14}C]xylose. In all the experiments performed no significant amount of [^{14}C]-D-xylose was incorporated without the addition of the bikunin peptide as acceptor. These results clearly indicate that a xylose residue was transferred to the hydroxyamino acid serine of the bikunin peptide through a β-linkage. In conclusion the expressed protein was identified as UDP-D-xylose:proteoglycan core protein β-D-xylosyltransferase (EC 2.4.2.26).

Table 3:

Xylosyltransferase activity of recombinant XT-I expressed in CHO-K1 cells. XT activity of the enzyme fractions using different acceptors and the inhibition of enzyme activity by addition of heparin is shown. The synthetic bikunin, L-APP and L-APLP2 homologous peptides have been previously proved to be good acceptors for XT mediated xylosylation (Brinkmann *et al.*, 1997; Götting *et al.*, 1998). No XT activity was detected in samples from mock-transfected cells after affinity purification. n.d., not detected (detection limit, 20 μU/l).

Acceptor	*XT Activitiy*	
	pCG227-XT	
		+ heparin
Recombinant bikunin	2854	n.d.
Bikunin peptide QEEGSGGGGQK	463	n.d.
L-APLP2 peptide SENEGSGMAEQK	515	n.d.
L-APP peptide TENEGSGLTNIK	492	n.d.
Peptide SGG	n .d.	n.d.
Chondroitin sulfate A	n.d.	n.d.
Chondroitin sulfate C	n.d.	n.d.

Tissue-specific expression of XT-I and the XT-II isoform

The expression of XT-I and XT-II isoform gene was examined in various human tissues using a RT-PCR based method with normalized first-strand cDNA. Each PCR yielded a single product with predicted nucleotide lengths of 490 bp for XT-I and 717 bp for XT-II, although the amount of the amplified product varied (Figure 11). Amplification of XT-I and XT-II fragments resulted in a product visible by ethidium bromide staining after 36 cycles, whereas the DNA fragment corresponding to the abundant glyceraldehyde-3-phosphate dehydrogenase transcript was visible after 20 cycles. The greatest abundance of XT-I expression was detected in the placenta, kidney and pancreas and only a very weak expression was detected in skeletal muscle. The greatest abundance of XT-II isoform is expressed in the kidney and pancreas.

Cellular distribution of XT activity in cultured CHO-K1 cells

After an incubation period of 3 days cultured CHO-K1 cells were harvested and the XT activity was determined in the spent culture supernatant and the cell lysates. 92% of the total XT activity was found to be located in the cell culture medium (93.1 mU/10^6 cells, SD 9.58), whereas only 2% was detected in the cell lysates (2.03 mU/10^6 cells, SD 0.44). 6% of the total XT activity (5.82 mU/10^6 cells, SD 2.18) was released from the membrane-containing fractions after addition of the detergence Triton X-100 indicating that less than 1/10th of XT is bound to cellular membranes.

Pharmaceutical and diagnostic use

The xylosyltransferase enzymes according to the invention can be used directly as therapeutic drug in pathological situations where a deficiency of said enzyme and its isoforms can be detected. As pointed out above XT enzyme may be overexpressed in some diseases and

disorders. In these cases inhibitors and / or antagonists of XT-I and or XT-II can be used. Thus, the invention relates also to the use for the manufacture of a medicament for the treatment of diseases which are caused by increased levels of said enzymes, wherein said medicament is an inhibitor or antagonist of xylosyltransferase.

As mentioned above the protein according to this invention can be used as diagnostic means to evaluate pathological conditions. As used herein, the term "pharmaceutically acceptable carrier" means an inert, non toxic solid or liquid filler, diluent or encapsulating material, not reacting adversely with the active compound or with the patient. Suitable, preferably liquid carriers, are well known in the art. The formulations according to the invention may be administered as unit doses containing conventional non-toxic pharmaceutically acceptable carriers, diluents, adjuvants and vehicles which are typical for parenteral administration.

The term "parenteral" includes herein subcutaneous, intravenous, intra-articular and intratracheal injection and infusion techniques. Also other administrations such as oral administration and topical application are suitable. Parenteral compositions and combinations are most preferably adminstered intravenously either in a bolus form or as a constant fusion according to known procedures. Tablets and capsules for oral administration contain conventional excipients such as binding agents, fillers, diluents, tableting agents, lubricants, disintegrants, and wetting agents. The tablets may be coated according to methods well known in the art.

Unit doses according to the invention may contain daily required amounts of the protein according to the invention, or sub-multiples thereof to make up the desired dose. The optimum therapeutically acceptable dosage and dose rate for a given patient (mammals, including humans) depends on a variety of factors, such as the activity of the specific active material employed, the age, body weight, general health, sex, diet, time and route of administration, rate of clearance, enzyme activity (units/mg protein), the object of the treatment, i. e., therapy or prophylaxis and the nature of the disease to be treated.

Therefore, in compositions and combinations in a treated patient (in vivo) a pharmaceutical effective daily dose of the protein of this invention (hXT, hXT-I, hXT-II) is between about 0.01 and 100 mg/kg body weight (based on a specific activity of 100 kU/mg), preferably between 0.1 and 10 mg/kg body weight. According to the application form one single dose may contain between 0.5 and 10 mg of hXT.

Short Description of the Figures

Figure 1: *Purification of XT*

Panel A, Heparin affinity chromatography on POROS 20 HE: The dissolved ammonium sulfate precipitate from JAR choriocarcinoma supernatant was applied to a POROS 20 HE column. After equilibration with buffer A, the column was eluted stepwise with NaCl (·····). Protein elution was monitored at wavelength A_{280} (——), and fractions of 38 ml were assayed for XT activity (■). The horizontal bracket indicates fractions collected for further purification.

Panel B, Ion exchange chromatography on POROS 20 HQ : The desalted and concentrated product from heparin affinity chromatography was loaded onto a POROS 20 HQ column. The column was washed with buffer A. Adsorbed proteins were eluted stepwise with NaCl (· · --). Protein elution was monitored at A_{280} (——), and fractions of 50 ml were assayed for XT activity (■). The horizontal brackets indicate the fractions collected for further purification.

Panel C, Protamine chloride affinity chromatography: The desalted and concentrated product from ion exchange chromatography was applied to a protamine chloride POROS column. After a washing step with buffer A, the column was eluted stepwise with NaCl (·····). Elution was monitored at A_{280} (——), and fractions of 6 ml were assayed for XT activity (■). The horizontal bracket indicates the fractions collected.

Figure 2 *SDS-PAGE of XT fractions at various purification steps*

Panel A: XT fractions at various purification steps were subjected to 4-12% gradient polyacrylamide gel for SDS-PAGE. *Lane I,* JAR cell culture supernatant (crude material); *lane II,* ultrafiltration retentate; *lane III,* dissolved ammonium sulfate precipitate; *lane IV,* protein eluted with NaCl from the POROS 20 HE column; *lane V,* protein eluted with NaCl from the POROS 20 HQ column; *lane VI,* protein eluted with NaCl from the protamine chloride POROS column. *Lane M,* molecular size standard: myosin (200.000), phosphorylase b (97.000), bovine serum albumin (66.000), glutamic dehydrogenase (55.000), carbonic anhydrase (31.000), trypsin inhibitor (22.000). The gel was silver-stained.

Panel B: Collected XT fractions from protamine affinity chromatography were subjected to SDS-PAGE on a 4-12% gradient polyacrylamide gel. The arrows indicate the stained bands corresponding to the unknown protein, which was shown to be XT (120.000) *(1),* hexose-6-phosphate dehydrogenase (89.000) *(2)*, ezrin (68.600) *(3),* quiescin Q6 (64.000) *(4)*, plasminogen activator (47.000) *(5),* aldolase A (39.000) *(6)* and low molecular artefacts *(7)*. All

protein bands were excised, enzymatically digested, and the peptide mixture was characterized by MALDI-TOF mass spectrometry. The gel was stained with Coomassie Brilliant Blue.

Figure 3: *Immunoaffinity chromatography of xylosyltransferase*

Panel A, 100 µl of the desalted XT-containing fractions eluted from heparin affinity matrix was applied to a column of immobilized polyclonal antibodies. After washing with buffer D, the column was eluted as indicated by the *arrow* with buffer D / 0.15 M NaCl (1) followed by 12 mM HCl (2). Fractions of 1 ml were collected into tubes containing 1 ml of 0.1 M Tris/HCl, pH 8.0. Protein elution was monitored at 280 nm (——) and the XT activities (■) of each fraction were assayed.

Panel B, Negative control of the immunoaffinity chromatography with immobilized preimmune serum. For conditions see *Panel A.*

Panel C, Immunoaffinity chromatography with immobilized polyclonal antibodies. The column was eluted with buffer D containing 100 µl (1 mg/ml) of the peptide antigen indicated by the arrow. Fractions of 1 ml were collected into tubes containing 1 ml of 0.1 M Tris/HCl, pH 8.0. Protein elution was monitored at 280 nm (——) and the XT activities (■) of each fraction were assayed.

Figure 4: *Aprotinin affinity chromatography of partially purified XT and immunoblot analysis of the separated fractions*

Panel A, 200 µl desalted XT fraction from the heparin purification step was applied to an aprotinin column previously equilibrated with buffer A. After washing with buffer A, the adsorbed proteins were eluted stepwise with NaCl (- - - -). Protein elution was monitored at A280 (——) and fractions of 2 ml were assayed for XT activity (■).

Panel B, Aliquots of fraction 3, fraction 7, fraction 12 and fraction 16 were analyzed by western blot. The MW 120.000 protein was detected in the XT-containing fraction 7 and 12. Immunological detections were performed using a polyclonal rabbit antiserum raised against the synthetic peptide CSRQKELLKRKLEQQEK deduced from the peptides 2 and 10 of the enzymatically cleaved unknown protein. Prestained molecular size standard were myosin (190.000), BSA (64.000), glutamic dehydrogenase (51.000).

Figure 5 : *Gel filtration chromatography and N-glycosidase F digestion*

Panel A, 100 µl of the XT containing fraction from heparin affinity HPLC was applied to a TSK G3000 SW column (30 cm X 7.5 mm, 10 µm particle size) previously equilibrated with buffer A / 0.15 M NaCl. Elution was performed with the same buffer. Fractions of 200 µl were collected and assayed for XT activity (■). Protein elution was monitored at A_{280} (——). The

arrows indicate the elution positions of thyroglobulin (669.000) *(1)*, ferritin (440.000) *(2)*, aldolase (158.000) *(3)*, albumin (67.000) *(4)*, ovalbumin (43.000) *(5)*, chymotrypsinogen A (25.000) *(6)*, and ribonuclease A (13.700) *(7)*.

Panel B, An aliquot of 1 µg of the MW 120.000 protein was analyzed by SDS-PAGE (I). Aliquots (1 µg) of the MW 120.000 protein were digested with 3,1 x 10^{-3} units of N-glycosidase F at 37°C for 1 h (II) and 12 h (III). The samples were then subjected to SDS-PAGE, and protein bands were detected by silver staining. The molecular size standard were myosin (200.000), b-galactosidase (116.000), phosphorylase b (97.000).

Figure 6: *Cloning strategy for human XT-I and XT-II isoform.*

(A) The peptides 4, 6, 7, and 9 are sequences that were most favorable for the design of degenerate PCR primers. The amino acid sequence of the peptides was obtained after proteolytic digestion of the purified human XT. The strategy for cloning of XT-I cDNA (B) and XT-II isoform (C) is illustrated. The open reading frame of XT-I and XT-II is shown as a filled box, and the location of the peptides 4, 6, 7 and 9 is illustrated by open boxes. The location and orientation of the degenerate primers employed for cloning of XT-I and XT-II are marked by arrows. XT-I and XT-II cDNA inserts contained within the indicated plasmids were obtained using RT-PCR with degenerate primers (pCG111-4, pCG114-29, pCG110-7), 5' RACE (pCG185-21, pCG212-19, pCG319-23, 3' RACE (pCG204-38, pCG211-4) and RT-PCR with gene-specific primers (pCG176-1). H = A + C + T, Y = C + T, R = A + G, N = A +G + C + T, I = deoxyinosine.

Figure 7A, B: *Nucleotide (A) and deduced amino acid (B) sequence of the hXT-I.* The position of the peptide sequences obtained by digestion of the purified human XT are underlined (B). Potential N-glycosylation sites are double underlined. The DxD motif is intensified depicted. The figures are identical with SEQ ID 1 and 2.

Figure 8A, B: *Nucleotide (A) and amino acid (B) sequence of the hXT-II isoform.* Potential N-glycosylation sites are double underlined (B). The putative transmembrane domain is underlined. The figures are identical with SEQ ID 3 and 4.

Figure 9: *Nucleotide sequence of rXT-I (SEQ ID 5).* The corresponding protein sequence is depicted in SEQ ID 6.

Figure 10: *Nucleotide sequence of rXT-II (SEQ ID 7).* The corresponding protein sequence is depicted in SEQ ID 8.

Figure 11: *Differential expression of the XT-I, XT-II gene in human tissues.* Semiquantitative RT-PCR with normalized first-strand cDNA was used to examine the abundance of XT-I and

XT-II transcripts. A 983 bp fragment of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase cDNA was amplified as control (A). The 490 bp XT-I cDNA fragment (B) and the 717 bp fragment amplified from XT-II isoform cDNA (C) were detected in each tissue indicating that both enzymes are ubiquitously expressed. The arrows indicate the expected position for each PCR product.

The following examples describe the invention in more detail without to limit scope of the technical teaching.

Example 1: *Materials for Isolation and Purifications.* Human JAR choriocarcinoma cells were purchased from ATCC (Rockville, MD). Dried UltraDOMA-PF medium was obtained from BioWhittaker (Vervier, Belgium) and *aqua ad injecta* from Braun (Melsungen, Germany). Heat-inactivated fetal calf serum, Dulbecco's phosphate-buffered saline, antibiotic/antimycotic solution, trypsin-EDTA solution, trypan blue, protamine chloride and the *Bicinchoninic Acid Protein Assay Kit* were purchased from Sigma (Deisenhofen, Germany). Cell culture flasks, serological pipettes, and sterile tubes were purchased from Becton Dickinson (Heidelberg, Germany). The hybrid hollow-fiber bioreactor TECNOMOUSE® was supplied by Integra Biosciences (Fernwald, Germany), the ACA analyzer by Dade Diagnostica (München, Germany) and the Super G analyzer by RLT (Möhnesee, Germany). UDP-[^{14} C]xylose (9.88 kBq / nmol) came from DuPont (Bad Homburg, Germany), 25 mm diameter nitrocellulose discs from Sartorius (Göttingen, Germany), scintillation mixture and the liquid scintillation counter LS500TD was obtained from Beckman Coulter (Fullerton, CA). Ultrafiltration cells, YM1 membranes and PVDF membranes (Immobilon P) were purchased from Millipore (Eschborn, Germany). The chromatography media POROS 20 HQ, POROS 20 HE2, POROS 20 AL, POROS 20 EP and the HPLC workstation *Biocad Sprint* were supplied by Perseptive Biosystems (Framingham, MA). The gel filtration column TSK G3000 SW (30 cm x 7.5 mm, 10 µm particle size) was obtained from TosoHaas (Montgomeryville, PA). The MALDI mass spectrometer Reflex II was from Bruker Daltonik GmbH (Bremen, Germany) and protein sequencer Procise 494 cLC was purchased from PE Biosystems (Framingham, MA). Precast polyacrylamide gels, buffers, and NuPAGE electrophoresis system XCell II Mini-Cell and Blot Module were from Novex (San Diego, CA). The synthetic peptide CSRQKELLKRKLEQQEK and the rabbit antiserum were purchased from BioScience (Göttingen, Germany). Peroxidase-conjugated affinipure F(ab')2 fragment goat anti-rabbit IgG (H+L) was purchased from

Dianova (Hamburg, Germany). N-glycosidase F was obtained from Roche (Mannheim, Germany).

Example 2: *Cell culture.* JAR choriocarcinoma cells releasing XT in the cell culture supernatant were cultured in Ultradoma-PF medium containing 10% heat-inactivated fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin and 250 ng/ml amphotericin B in a humidified atmosphere of 5% CO2 and 95% air at 37°C. After incubation for 24 h in the serum-containing medium, the cell cultivation was adapted to serum-free conditions as described previously (6). Scaling up of XT production was carried out in three hybrid hollow-fiber bioreactors TECNOMOUSE®. During the exponential growth the cells from three 175 cm 2 T-flasks (> 3 x 10^7 cells) were detached with 0.5% trypsin and 0.2% EDTA in Dulbecco's phosphate-buffered saline by incubation at 37°C for 10 min. After centrifugation (5 min, 1000 x g) of the cell suspension the cell pellet was resuspended in 10 ml 37°C warm serum-free and protein-free Ultradoma-PF medium and washed three times with 20 ml of the same medium. The cell suspension was drawn into a 10 ml syringe and then inoculated into the extracapillary space (EC space) of the reactor. The bioreactor was connected with a 2 liter medium bottle and set to 150 ml/h in the recirculation mode, and the oxygenation pump was set as described in the operating manual. Five days after inoculation a 10 ml syringe was connected to the left hand EC port and 10 ml of cell culture supernatant was harvested from the EC space. The harvesting was continued every two days over a period of 3 months. Glucose and lactate concentration of the cell culture supernatant were controlled using the Super G analyzer and the ACA analyzer, respectively. The 2 liter medium bottle was replaced every three days. The viability of the cells was determined by trypan blue exclusion.

Example 3: *Synthesis of the protamine affinity matrix.* Protamine chloride was immobilized as ligand on POROS 20 AL. 30 mg ligand was dissolved in 10 ml of 10 mM phosphate / 0.15 M NaCl, pH 7.4. After the protein had been dissolved, 5 ml of 100 mM phosphate / 1.50 M NaCl, pH 7.4 was added. $NaCNBH_3$ was dissolved in the ligand/buffer solution to a final concentration of 5 mg/ml and 1.0 g POROS 20 AL was suspended in the same solution. The suspension was mixed gently on a shaker for 1 minute at RT. An additional 2 ml of 100 mM phosphate / 1.50 M NaCl, pH 7.4 was added to the suspension and the mixture was shaken continuously. This step was repeated every five minutes until the mixture volume was 25 ml. After additional shaking for 2 h, the medium was filtered on a sintered glass funnel. The matrix was suspended in 20 ml of 0.2 M Tris/HCl / 5 g/l $NaCNBH_3$, pH 7.2 and mixed gently on a shaker for 30 min at RT. After the media had been washed in a sintered glass funnel using 100

ml of 10 mM phosphate, pH 7.4, 100 ml of 1.0 M NaCl and another 100 ml of 10 mM phosphate, pH 7.4, the matrix was packed in a PEEK column (4.6 x 50 mm).

Example 4: *Synthesis of the aprotinin affinity matrix.* Aprotinin affinity matrix was synthesized according to the synthesis of the protamine affinity matrix, but using aprotinin as ligand. After immobilization of the ligand the matrix was packed in a PEEK column (4.6 x 50 mm).

Example 5: *Purification of xylosyltransferase from JAR cell culture supernatant.*

Fractionated ammonium sulfate precipitation and chromatography steps were performed at RT, ultrafiltration and diafiltration were carried out at 4°C. 18.5 liters JAR cell culture supernatant collected from three hybrid hollow-fiber bioreactors, TECNOMOUSE®, each containing 5 culture cassettes, was concentrated to 800 ml with ultrafiltration cells using YM1 cellulose membranes. The retentate was centrifuged at 4,000 x *g* for 1 h. The supernatant was decanted, and the pellet was discarded.

Step 1: Fractionated ammonium sulfate precipitation - Solid ammonium sulfate was added to the supernatant to 28% saturation. After 1 h at RT the suspension was centrifuged at 4,000 x *g* for 2 h, the supernatant was decanted, and the precipitate was removed. Additional ammonium sulfate was added to the solution to the point of 40% saturation, and the suspension was allowed to stand for 1 h. To recover the precipitate the supernatant was decanted after the suspension was centrifuged at 4,000 x *g* for 2 h. Before chromatography on immobilized heparin the precipitate was dissolved in 460 ml buffer A (20 mM sodium acetate, pH 6.0).

Step 2: Heparin affinity chromatography on POROS 20 HE2 - The step 1 product was passed through a 0.2 µm filter. 4.0 ml of the filtrate was applied to a POROS 20 HE2 column (16 x 100 mm) equilibrated with buffer A at a flow rate of 40 ml/min. After washing the column with 100 ml of buffer A the XT activity was eluted with the same buffer containing NaCl. The NaCl concentration was increased stepwise: 20 ml buffer A / 0.09 M NaCl; 20 ml buffer A / 0.15 M NaCl; 30 ml buffer A / 0.24 M NaCl; 24 ml buffer A / 0.30 M NaCl; 24 ml buffer A / 0.60 M NaCl; 24 ml buffer A / 1.00 M NaCl; and 24 ml buffer A / 1.89 M NaCl. Fractions of 38 ml each were collected and the XT activity was measured. The procedure was repeated 115 times by cyclic chromatography and the fractions containing XT activity (115 x 38 ml) were collected.

Step 3: Ion exchange chromatography on POROS 20 HQ - Collected fractions from step 2 were desalted using diafiltration with YM1 cellulose membranes and ultrafiltration cells. After concentration of the desalted protein solution to 0.05 liter using analogous techniques the XT-enriched solution was subjected to ion exchange chromatography. 4.0 ml of the XT solution

was applied onto the POROS 20 HQ column (16 x 100 mm) previously equilibrated with buffer A at a flow rate of 40 ml/min. The column was washed with 80 ml buffer A, and the adsorbed protein was eluted stepwise using the same buffer containing 0.07 M NaCl (88 ml), 0.18 M NaCl (120 ml), and 0.36 M NaCl (120 ml) followed by a linear gradient of 0.36 – 1.00 M NaCl (200 ml) and another step of buffer A / 2.0 M NaCl (120 ml). 50 ml fractions were collected and assayed for activity and evaluated by SDS-PAGE. Chromatography was repeated 13 times, and the fractions exhibiting XT activity (13 x 50 ml) were collected for affinity chromatography.

Step 4: Affinity chromatography on protamine chloride - XT-containing solution from step 3 was desalted as described above and concentrated to 5 ml by ultrafiltration with YM1 cellulose membranes. The ultrafiltration product was passed through a 0.2 µm filter. 100 µl of the filtrate was loaded onto a protamine chloride - POROS column (4.6 x 50 mm) equilibrated with buffer A. The flow rate was 10 ml/min. The column was washed with 10.0 ml of buffer A, and the adsorbed fraction was eluted with the same buffer containing NaCl by a stepwise increase of the NaCl concentration: 6.6 ml buffer A / 0.04 M NaCl; 6.6 ml buffer A / 0,06 M NaCl; 6.6 ml buffer A / 0.23 M NaCl followed by a linear gradient of 0.23 – 1.20 M NaCl (4.2 ml) in buffer A. Fractions of 6.0 ml were collected, assayed for XT activity and evaluated by SDS-PAGE. Cyclic chromatography was repeated 50 times. The purified enzyme was collected, concentrated to 1.0 ml using ultrafiltration techniques and stored at – 75 °C.

Step 5: SDS-Polyacrylamide gel electrophoresis (SDS-PAGE) - The protein composition of various fractions was estimated by SDS-PAGE. Briefly, 12.1 µl of sample was added to 4.7 µl of sample buffer (1.00 M Tris/HCl / 1.17 M sucrose, 0.28 M SDS, 2.08 mM EDTA, 0.88 mM Serva Blue G250, 0.70 mM phenol red, 0.10 M DTT, pH 8.5) and heated for 10 minutes at 99°C. After the sample had been loaded, SDS-polyacrylamide gel electrophoresis was carried out on a 4-12% bis-tris polyacrylamide gel with 3-(N-morpholino) propanesulfonic acid (MOPS) running buffer (1.00 M MOPS/Tris / 69.3 mM SDS, 20.5 mM EDTA, pH 7.7). Protein bands were detected by Coomassie Brilliant Blue or by silver staining. The Coomassie bands were excised and characterized by MALDI mass spectrometry and amino acid sequence analysis.

Example 6: *MALDI mass spectrometry.* Coomassie-stained proteins were excised from the gel, repeatedly washed with H_2O and H_2O/acetonitrile and digested overnight with trypsin and endoproteinase Lys-C at 37°C. The peptides generated in the supernatant were analyzed by MALDI mass spectrometry. Sample preparation was achieved following the thin film

preparation techniques (13). Briefly, aliquots of 0.3 µl of a nitrocellulose containing saturated solution of a-cyano-4- hydroxycinnamic acid in acetone were deposited onto individual spots on the target. Subsequently, 0.8 µl 10% formic acid and 0.4 µl of the digest sample was loaded on top of the thin film spots and allowed to dry slowly at ambient temperature. To remove salts from the digestion buffer the spots were washed with 10% formic acid and with H_2O. MALDI mass spectra were recorded in the positive ion mode with delayed extraction on a Reflex II time-of-flight instrument equipped with a SCOUT multiprobe inlet and a 337 nm nitrogen laser. Ion acceleration voltage was set to 20.0 kV, the reflector voltage was set to 21.5 kV and the first extraction plate was set to 15.4 kV. Mass spectra were obtained by averaging 50-200 individual laser shots. Calibration of the spectra was performed internally by a two-point linear fit using the autolysis products of trypsin at m/z 842.50 and m/z 2211.10.

Example 7: *Amino acid sequence analysis of XT.* The MW 120.000 Coomassie-stained protein was excised from the gel, repeatedly washed with H_2O and H_2O/acetonitrile and digested with trypsin and endoproteinase Lys-C overnight. For HPLC separation the excised gel fragment was extracted twice with 0.1% TFA/60% acetonitrile. The extracted enzymatic fragments were separated on a capillary HPLC system equipped with a 140B solvents delivery system (PE Biosystems), Acurate splitter (LC-Packings), UV absorbance detector 759A (PE Biosystems), U-Z capillary flow cell (LC-Packings) and Probot fraction collector (BAI) using reversed-phase column (Hypersil C18 BDS, 3 µm, 0.3 x 150 mm) and a linear gradient from 12% acetonitrile, 0.1% TFA to 64% acetonitrile, 0.08% TFA in 90 min with a flow rate of 4 µl/min at RT. Peptide elution was monitored at 214 nm and individual fractions from the HPLC separation were analysed by MALDI mass spectrometry. Sequence analysis of separated fragments was performed on a Procise Protein Sequencer 494 cLC using standard programs supplied by PE Biosystems.

Example 8: *Determination of XT activity.* Determination of XT activity is based on the incorporation of [14 C]-D-xylose into the recombinant bikunin according to a previously described method (Brinkmann et al., 1997, J. Biol. Chem., 272, 11171-11175). For analysis of the substrate specificity of the recombinant xylosyltransferases synthetic peptides containing the XT recognition sequence were used as acceptor. The reaction mixture for the assay contained, in a total volume of 100 µl: 50 µl of XT solution, 25 mM 4-morpholineethanesulfonic acid (pH 6.5), 25 mM KCl, 5 mM KF, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 1.0 µM UDP-[^{14}C]-D-xylose, and 1.5 µM of the synthetic peptides. After incubation for 75 min at 37°C, the reaction mixtures were placed on discs (25 mm diameter) of Immobilon-

AV membrane, which immobilizes even small peptides by covalent links (Pfund & Bourdage, 1990, *Mol. Immunol.* 27, 495-502), and allowed to dry. It was then washed for 10 min with 10% trichloroacetic acid and three times with 5% trichloroacetic acid solution. Incorporated radioactivity was determined by liquid scintillation counting.

Example 9: *Antiserum Preparation.* The synthetic peptide CSRQKELLKRKLEQQEK deduced from the sequenced peptides 2 and 10 of the enzymatically cleaved XT was synthesized, purified by HPLC and used for immunization of rabbits (BioScience, Germany). Polyclonal antiserum was obtained by injection of the above antigen followed **Example 10:**

Preparation of solid-phase antigen. The antigen CSRQKELLKRKLEQQEK was immobilized on POROS 20 EP. After 1.6 mg antigen was dissolved in 1.2 ml of 10 mM phosphate / 0.15 M NaCl, pH 7.4 0.60 ml of 100 mM phosphate / 1.50 M NaCl, pH 7.4 was added. 400 mg POROS 20 EP was suspended in the solution and the suspension was mixed gently on a shaker at RT. At 10-min intervals, five times in total, an additional 0.24 ml of 100 mM phosphate / 1.50 M NaCl, pH 7.4 was added to the suspension. After additional shaking for 5 days at RT the suspension was filtered on a sintered glass funnel. The matrix was suspended in 4 ml 0.2 M phosphate / 0.1 M 2-mercaptoethanol, pH 7.4 and mixed on a shaker for 2 h at RT. The matrix was washed in a sintered glass funnel using 20 ml of 10 mM phosphate / 0.15 M NaCl, pH 7.4 and 20 ml of 1.0 M NaCl. After additional washing with 20 ml of 10 mM phosphate, pH 7.4, the matrix was packed in a PEEK column (4.6 x 50 mm).

Example 11: *Antibody purification.* Antiserum was adjusted to 50 mM Tris/HCl, pH 8.0. The solution was clarified by passage through a 0.2 µm filter. 0.4 ml filtrate was applied at 10 ml/min to the antigen column previously equilibrated with buffer B (50 mM Tris/HCl, pH 8.0). After the column was washed with 4.1 ml buffer B and with 12.5 ml buffer B / 0.15 M NaCl, the adsorbed antibody was eluted using 12.4 ml of 50 mM sodium citrate / 0.15 M NaCl, pH 3.0 followed by 3.4 ml of 100 mM sodium citrate / 1.5 M NaCl, pH 3.0. The eluate was collected as 10 ml fractions in tubes containing 2 ml of 0.5 M Tris/HCl, pH 8.0, to immediately neutralize the citric acid.

Example 12: *Preparation of immunoaffinity column.* Purified antibody was concentrated to a protein concentration of 0.3 mg/ml using ultrafiltration with YM1 cellulose membranes. The antibody solution was adjusted to 10 mM phosphate / 0.15 M NaCl, pH 7.4. After filtration of the solution through a 0.2 µm filter 100 µl filtrate was applied at 0.2 ml/min to a POROS 20 PA column (2.1 x 30 mm). This step was repeated 17 times. Adsorbed antibody was cross-linked using cross-linking solution (100 mM triethanolamine, pH 8.5). After the column was

washed with 5 ml of buffer C (10 mM phosphate / 0.15 M NaCl, pH 7.4) 2 ml cross-linking solution was applied at 0.5 ml/min onto the cartridge. The procedure was repeated 6 more times, using a total volume of 14 ml cross-linking solution. To block unreacted functional groups on the cross-linking reagents 2 ml of 100 mM monoethanolamine, pH 9.0 (quenching solution), was loaded onto the cartridge at 0.5 ml/min. The column was washed using 2 ml of buffer C and the cross-linking step was repeated using another 2 ml quenching solution. The immunoaffinity column was cycled between buffer C and 12 mM HCl / 0.15 M NaCl 3 times using a total volume of 12 ml of solution.

Example 13: *Immunoaffinity column purification of XT.* XT-containing fractions eluted from the heparin affinity matrix were desalted using diafiltration with YM1 cellulose membranes and passed through a 0.2 µm filter. 100 µl of this XT-sample was applied to the immunoaffinity column equilibrated with buffer D (20 mM Tris/HCl, pH 8.0) at a flow rate of 1 ml/min. The column was washed with 1.4 ml of buffer D and with 8.5 ml of buffer D / 0.15 M NaCl. The XT activity was eluted with 4.2 ml of 12 mM HCl followed by 1.2 ml of 12 mM HCl / 1.5 M NaCl. Alternatively the elution was performed using 100 µl of antigen at 1 mg/ml in buffer D. Fractions (1 ml) were collected into tubes containing 1 ml of 0.1 M Tris/HCl, pH 8.0. The XT activities of the fractions were determined.

Example 14: *Aprotinin affinity chromatography.* 200 µl of desalted XT solution from the heparin purification step was applied at 10 ml/min to the aprotinin column previously equilibrated with buffer A. After washing the column with 6.6 ml of buffer A the adsorbed protein was eluted stepwise using the same buffer containing 0.3 M NaCl (10.0 ml), 0.54 M NaCl (10.0 ml), 1.00 M NaCl (10.0 ml) and 1.50 M NaCl (2.4 ml). **Example 15:** *Western Blot Analysis.* For western blot analysis, proteins were transferred to polyvinylidene difluoride membrane in a semi-dry instrument (Novex). After transfer nonspecific antibody binding sites were blocked with 2% BSA in 0.1 M Tris/HCl, pH 7.2, for 1 h at RT. The membrane was incubated with antiserum in 50 mM phosphate / 0.15 M NaCl, 0.5 ml/l Tween 20, pH 7.4 at 1:1000 dilution for 1 h. Bound antibody was detected using a second anti-rabbit goat immunoglobulin coupled to horse-radish peroxidase at a 1:1000 dilution. The blot was developed using 4-chloro-1-naphthol.

Example 16: *Gel filtration chromatography.* A sample of 100 µl from the heparin purification step was applied at 1.0 ml/min to a TSK G3000 SW column (30 cm x 7.5 mm, 10 µm particle size) which had previously been equilibrated with buffer A / 0.15 M NaCl. Proteins were eluted with the same buffer. Fractions of 200 µl were collected and tested for XT activity.

Column calibration was performed using thyroglobulin (669.000), ferritin (440.000), aldolase (158.000), albumin (67.000), ovalbumin (43.000).

Example 17: *N-glycosidase F digestion.* Aliquots (1 µg) of XT were digested with 3,1 x 10^{-3} units of N-glycosidase F at 37°C for 1 h and 12 h (Table II). The samples were then subjected to SDS-PAGE, and protein bands were detected by silver staining.

Example 18: *Measurement of protein concentration.* Protein concentration was estimated by absorbance at 280 nm assuming $E^{1\%}_{1\,cm} = 10.0$ or with the *Bicinchoninic Acid Protein Assay* using bovine serum albumin as a standard.

Example 19: *PCR-based cloning of human xylosyltransferase.* Degenerate oligonucleotide primers with deoxyinosine substitution were designed based upon the amino acid sequence of peptides obtained after digestion of the isolated human XT with trypsin or Lys-C. The first strand of cDNA was synthesized by the reverse transcription reaction using poly(A)$^+$ RNA isolated from the chondrosarcoma cell line SW1353 as template and oligo(dT) as primer. The reverse transcription reaction was performed at 37°C for 2 h using 50 pmol of oligo(dT) primers, 1 µg of poly(A)$^+$ RNA, a 0.5 mM concentration of each dNTP, 1x RT buffer and 200 units of RNase H deficient Moloney murine leukemia virus reverse transcriptase (Life Technologies, Eggenstein, Germany) in a final volume of 20 µl. For PCR amplification the reaction mixture contained 4 µl of the reverse transcription reaction solution, 50 pmol of each primer, a 0.25 mM concentration of each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$ and 2.5 units of hot start *Taq* polymerase (Life Technologies) in a final volume of 50 µl. Amplification with degenerate oligonucleotide primers was carried out by 40 cycles at 94°C for 1 min, 48°C for 1 min, and 72°C for 2 min, followed by a final extension step at 72°C for 15 min. After agarose electrophoresis of the PCR products, DNA fragments were excised, subcloned into the pCR2.1 vector (Invitrogen. Groningen, Netherlands) and sequenced by the dideoxy chain termination method using *Taq* DNA polymerase (Big-dye terminator cycle sequencing kit, Perkin-Elmer, Norwalk, CT, USA) with an automated DNA sequencer (Applied Biosystems, Weiterstadt, Germany). Several clones were sequenced to compensate for misreading by *Taq* polymerase.

Example 20: *Rapid amplification of 5' and 3' cDNA ends (RACE).* For amplification of the 5' and 3' ends of the XT-I and XT-II isoform cDNA RACE experiments were performed using commercially available systems (Clontech, Heidelberg, Germany; Life Technologies) according to the manufacturers' instructions. For 3'-RACE 1 µg placenta poly(A)$^+$ mRNA (Clontech) was reverse transcribed with a 3'-CDS primer (Clontech). PCR amplification of the 3' cDNA end of

human XT-I was accomplished according to a touch-down PCR protocol (5 cycles: 94°C for 30 s, 72°C for 3 min, 5 cycles: 94°C for 30 s, 70°C for 45 s, 72°C for 3 min, 25 cycles: 94°C for 30 s, 65°C for 45 s, 72°C for 3 min) with the gene-specific primer GSP1b 5'-GTGGGTATGCAGAAGTGGGGGAAGGGAC-3' and the UPM primer mix (Clontech). An aliquot of the first PCR was subjected to semi-nested PCR (30 cycles) using the primer Con2111 5'-CCCTCCGCAATGCCTACA-3' and the UPM primer mix. The DNA fragments obtained were subcloned into the vector pCR2.1 (Invitrogen) and sequenced. PCR amplification of the 3' cDNA end of the XT-II isoform was carried out with the primers AB10267 5'-ACTGAGGTCACGCAATACAA-3' and UPM using the touch-down PCR protocol. For 5'-RACE 1 µg of placenta poly(A)$^+$ mRNA was subjected to a reverse transcription and 5' tailing reaction with the 5'-CDS primer and the SMART oligo (Clontech) according to the manufacturer's protocol. The 5' end of the XT-I cDNA was amplified using the primer GSP_776L 5'-GCCGCACTCAG-GTGATGAAGAAGT-3' and UPM with a touch-down PCR protocol. An aliquot was used as template in a second semi-nested PCR reaction with the primers GSP_503L 5'-ACCA-CCAGGACAAAGGCGATTCTGA-3' and UPM. The 5'-cDNA end of XT-II was obtained by 5'-RACE amplification using the primers AB10315L 5'-AGTCGAACAGTCCAGG-GCCC-3' and UPM mix. A new primer for a 5'-RACE reaction was designed based upon the nucleotide sequence of the largest fragment obtained (2 kbp), and the experiment was repeated with the primer AB5846L 5'-CACGATCTCGCACTTGGGGG-3' as described above. The nucleotide sequences of human XT-I and XT-II cDNA have been submitted to the GenBank/EBI Data Bank with the accession numbers AJ277441 and AJ277442.

Example 21: *Isolation of XT-I cDNA from human brain and chondrocyte cDNA libraries.* Plasmid-DNA was isolated from a human whole brain cDNA library (Life Technologies) and a human chondrocyte cDNA library (Clontech) and used as template in a PCR-based approach for isolation of the 5' end of the XT-I cDNA. Briefly, GSP_776L and 5'ADLD 5'-CTATTCGATGATGAAGATACCCCACCAAACCC-3' primers were used in a PCR reaction with 1 µg of plasmid DNA as template. The DNA fragments were subjected to agarose gel electrophoresis and 0.7-3 kb long fragments were excised from the gel and used as a template in a semi-nested PCR reaction with GSP_503L and 5'-ADLD primers. DNA fragments were then subcloned into the vector pCR2.1 and sequenced.

Example 22: *Cloning of rat XT-I and XT-II cDNA.* A RT-PCR based approach using primers based upon the nucleotide sequence of human XT-I and XT-II was employed for amplification of XT-I and XT-II cDNA from rat. The first strand of cDNA was synthesized by the reverse

transcription reaction using poly(A)$^+$ RNA isolated from the rat liver cell line BRL3A as template and oligo(dT) as primer. DNA fragments obtained after PCR amplification using moderate stringent conditions were subcloned into the vector pCR2.1 and sequenced. The 5' and 3' ends of the XT-I and XT-II cDNA from rat tissue were amplified using the RACE strategy with gene-specific primers as described above. The nucleotide sequences of rat XT-I and XT-II cDNA have been submitted to the GenBank/EBI Data Bank with the accession numbers AJ295748 and AJ295749.

Example 23: *Expression levels of the XT-I and the XT-II isoform in human tissues.*

A Human Multiple Tissue cDNA Panel (Clontech) was used for the analysis of expression levels. Levels of amplification of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase, whose transcript is always present in the tissues at an almost constant level, were determined in parallel for quality control. For amplification of the XT-I encoding transcript the primers 128U and 601L were used, whereas primers AB10267U and AB12394L 5'-GGAAGAGCTGGGTGTGGAAT-3' were employed for the XT-II. PCR reactions were carried out by 22-36 cycles at 94°C for 30 s, 60-65°C for 45 s and 72°C for 2 min. Amplification of a transcript was performed using a serial number of cycles to find the conditions for semiquantitative amplification, and aliquots were analyzed by agarose gel electrophoresis.

Example 24: *Transfection and transient expression of XT.* For construction of a eukaryotic expression vector a DNA fragment including the known coding sequence of XT-I cDNA was amplified by PCR using XT_Exp1L 5'-TTTCCCGTTGAGATCCTGCT-3' and XT_Exp3U 5'-ACAGACAGCAACAACGAGAA-3' as primers and placenta first-strand cDNA (Clontech) as template. The 2450 bp fragment obtained was cloned into the vector pcDNA4/HisMax-TOPO (Invitrogen) resulting in the fusion of XT-I to the *Xpress* epitope. The plasmid pCG227-XT-I was then transiently transfected into CHO-K1 cells. The coding region of the XT-II cDNA was amplified by PCR using the primers AB_Exp9U 5'-AAAGGAAGGCAGAGGAAGC-3' and AB_Exp3L 5'-ACCCCTCCACTGT-CTGTAAG-3' and placenta first-strand cDNA as template. The obtained 2440 bp DNA fragment was cloned into the expression vector pcDNA4/HisMax-TOPO resulting in the fusion of XT-II to the aminoterminal *Xpress* epitope. The plasmid termed pCG226-XT-II was transiently transfected into CHO-K1 cells. $2x10^5$ cells precultured for 1 day in a 35 mm diameter cell culture dish were transfected with 2 µg of plasmid DNA and 6 µl of Fugene 6 transfection reagent (Roche, Mannheim, Germany). For determination of transfection efficiency CHO-K1 cells were transfected with 2 µg of the control plasmid pcDNA4/HisMax-TOPO-*lacZ*. 48 h after transfection the cell culture medium was harvested.

Protein G agarose beads (Sigma, Deisenhofen, Germany) and mouse anti-*Xpress* monoclonal antibody (Invitrogen) were added to the cell culture supernatant and incubated at 4°C for 1 h. After centrifugation at 10.000 g for 1 min the absorbed proteins were twice washed with PBS and resuspended in a final volume of 50 µl. XT activity was then assayed in the samples.

Example 25: *Characterization of the reaction products.* For characterization of the reaction product of recombinant XT-I the peptide QEEEGSGGGQK, which is homologous to the amino terminus of bikunin (Brinkmann *et al.*, 1997, l.c.), was used as acceptor in the XT activity assay. After incubation for 75 min at 37°C the enzyme was heat-inactivated by incubation for 15 min at 65°C and the reaction mixture was used for α- and β-xylosidase treatment and alkaline β-elimination. For the linkage-specific digestion of the bound [^{14}C]-D-xylose 4 mU of α-xylosidase (Seikagaku Corporation, Tokyo, Japan) or 4 mU of β-xylosidase (Sigma, Dreieich, Germany) were added to the samples and incubated for 60 min at 37°C. The reaction mixtures were then placed on Immobilon-AV membrane discs and allowed to dry. The discs were washed with trichloroacetic acid as described above and the remaining incorporated radioactivity was determined against appropriate controls. The alkaline cleavage of the O-glycosidic linkage between the [^{14}C]-D-xylose and the β-hydroxyamino acid serine was performed as described elsewhere (Montreuil *et al.*, 1994). Briefly, the reaction mixture was adjusted to pH 10 with diluted NaOH and an equal volume of cold, freshly prepared sodiumboro-hydride solution (2 M sodiumborohydride in 0.1 M NaOH) and was added. After incubation at 45°C for 16 h the cooled solution was neutralized by adding 50% acetic acid and placed on Immobilon-AV membrane discs. After drying the discs were washed with trichloroacetic acid and the remaining radioactivity was measured by liquid scintillation counting.

Patent Claims

1. An isoform of *UDP-D-xylose:proteoglycan core protein ß-D-xylosyltransferase*(XT).

2. A protein comprising an amino acid sequence of the isoform of claim 1 or a portion thereof, having the biological activity of XT.

3. A protein of claim 1 or 2 deriving from human or rat sources (hXT, rXT)

4. An isolated protein according to claim 3, wherein said hXT has a molecular weight of 120.000 under SDS polyacrlylamide gel electrophoresis conditions.

5. A recombinant protein of claim 3, wherein said protein is hXT-I comprising at least 827 amino acids.

6. A protein according claim 5, having the amino acid sequence as depicted in Fig. 7B.

7. A protein according to claim 3, wherein said protein is hXT-II comprising 865 amino acids and is an isoform of hXT-I.

8. A protein according to claim 7 exhibiting approximately 55% overall sequence identity to hXT-I

9. A protein according claim 8, having the amino acid sequence as depicted in Fig. 8B.

10. A DNA sequence coding for a protein of any of the claims 1 – 9.

11. A DNA sequence according to claim 10 comprising the nucleotide sequence coding for hXT-I as depicted in Fig. 7A, or rXT-I as depicted in Fig. 9.

12. A DNA sequence according to claim 10 comprising the nucleotide sequence coding for hXT-II as depicted in Fig. 8A or rXT-II as depicted in Fig. 10.

13. An expression vector comprising a promotor sequence, a DNA sequence of claim 10 and optionally a signal sequence.

14. An expression host cell comprising a vector of claim 13, said host cell being capable of expressing a protein of any of the claims 1 – 9.

15. An antibody directed against a protein as defined in any of the claims 1- 9.

16. A process for isolating and purifying a protein as defined in claims 1 – 4, characterized by the following steps:

(i) culturing cells having an increased level of XT, and harvesting the supernatant of said cell culture,

(ii) fractionated ammonium sulfate precipitation of the supernatant of step (i),

(iii) heparin affinity chromatography of the precipitate of step (ii),

(iv) ion exchange chromatography of the step (iii) product,

(v) affinity chromatography of the step (iv) product, and

(vi) SDS-Polyacrylamide gel elektrophoresis of step (v).

17. A pharmaceutical composition comprising a protein of any of the claims 1 – 9 and a pharmacologically acceptable carrier, diluent or excipient.

18. Use of a protein of any of the claims 1 – 9 for the manufacture of a medicament for the treatment of sclerotic diseases and chronic inflammatory joint diseases.

19. Use of claim 18, wherein said medicament is an inhibitor or antagonist of said protein.

20. Use of a protein of any of the claims 1 – 9 as diagnostic marker.

21. Use of a DNA molecule of any of the claims 10 – 12 as gene marker.

22. Use of an antibody as defined in claim 15 in an immunological assay for determination of a protein having the biological activity of hXT as diagnostic tool.

Fig. 1

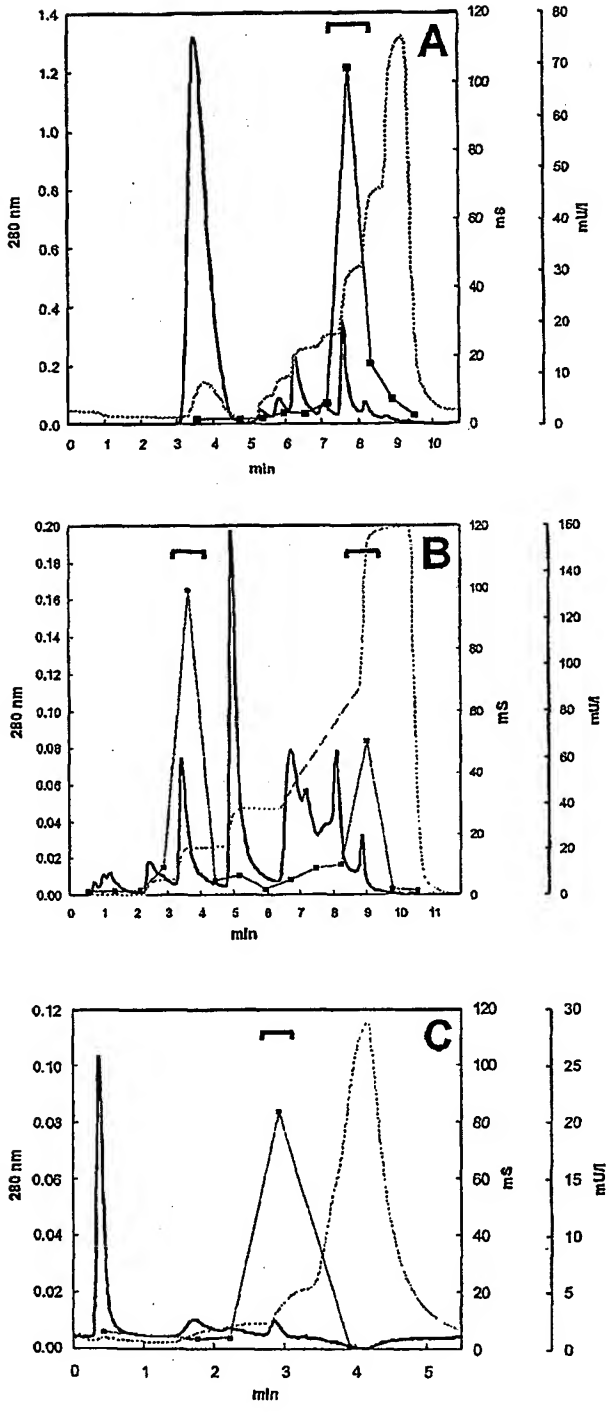


Fig. 2

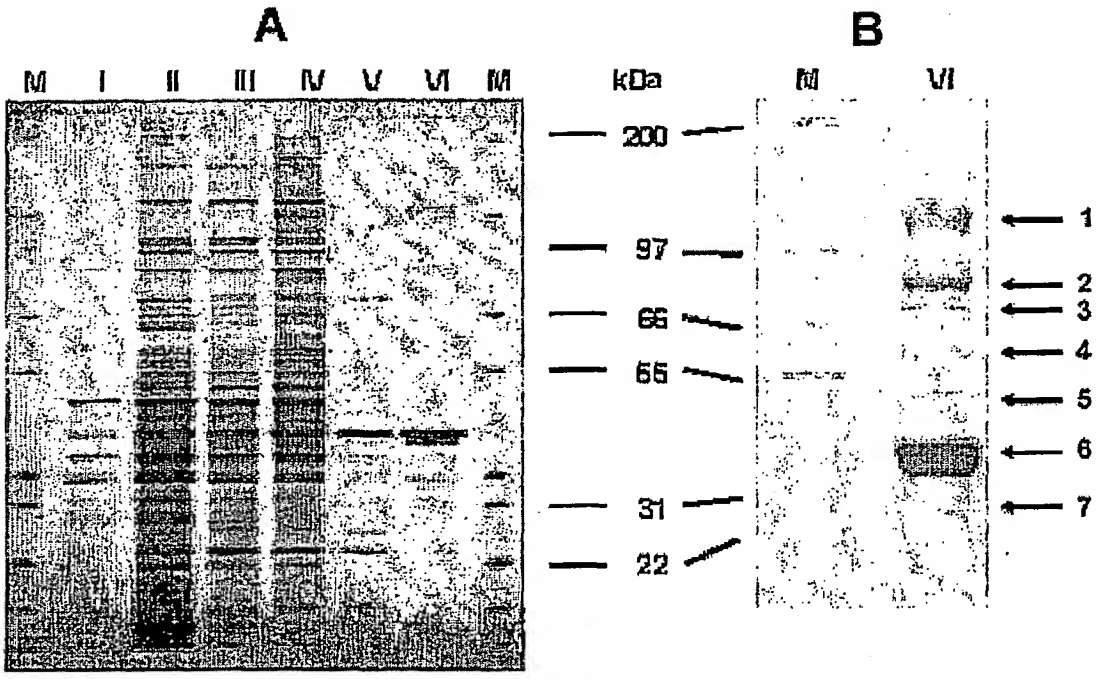


Fig. 3

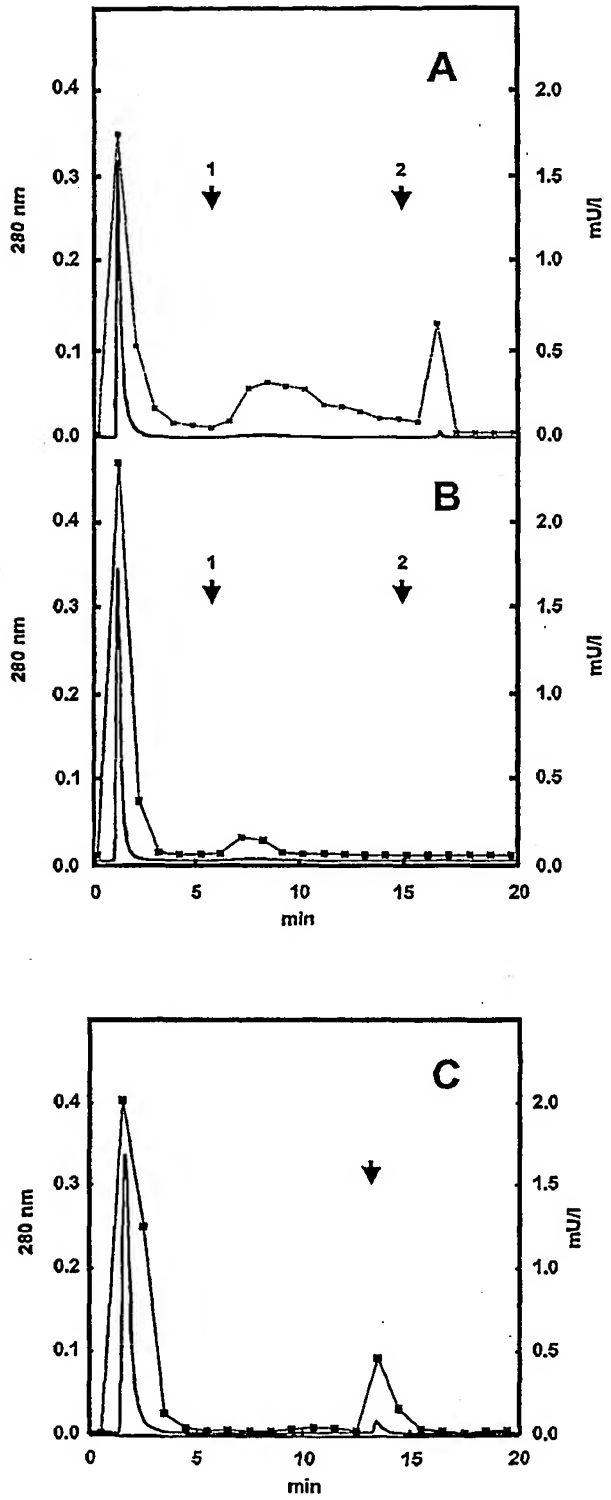
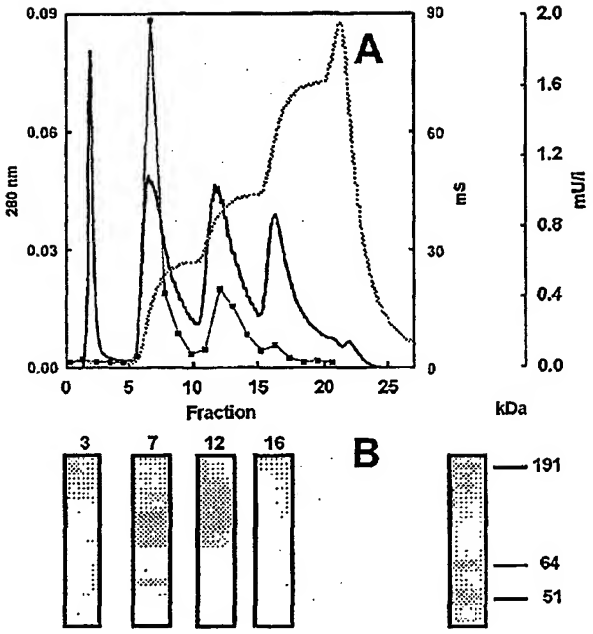


Fig. 4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0.4
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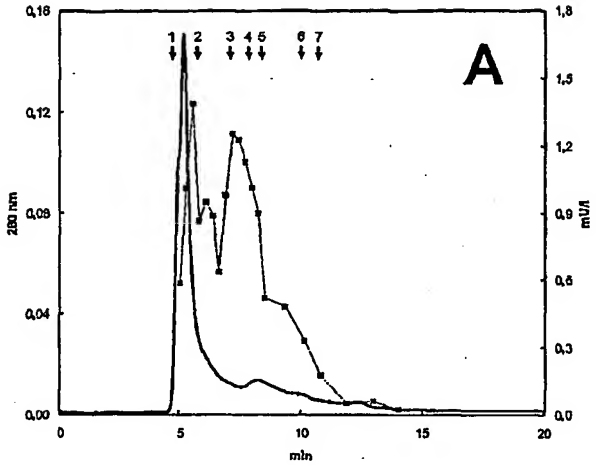
Fig. 5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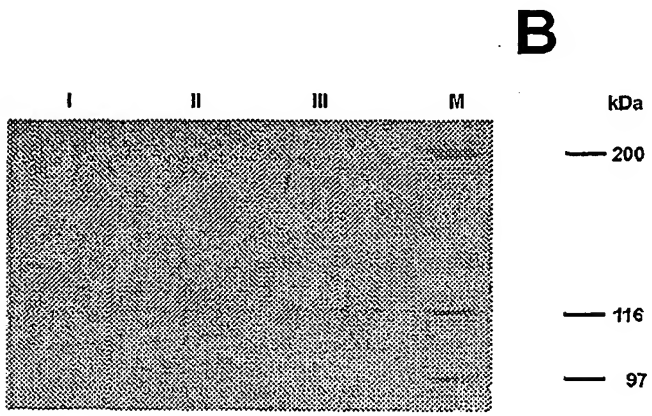
B
I
II
III
M
kDa
200
116
97

Fig. 6

Ⓐ

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SPPS1        5'-ATH GCI AGY CCI CCI AGY GA-3'
Peptide 4       Ile Ala Ser Pro Pro Ser Asp Phe Gly Arg
Inv2b        5'-TAD CGN AGI GGN GGI AGI CT-3'
Inv2c        5'-TAD CGN TCR GGI GGI TCR CT-3'

DF1         5'-GAY TTY GAR AAY GTI GAY AA-3'
Peptide 6      Asp Phe Glu Asn Val Asp Asn Ser Asn Phe Ala Pro Arg

PFF-sense   5'-CCI ACI TTY TTY GCN CG-3'
Peptide 7      Pro Thr Phe Phe Ala Arg

Peptide 9      Glu Leu Gly Ala Val Lys Pro Asp Gly Arg Leu
Lysc-inv1                3'-CCN CGI CAN TTY GGN CTR CC-5'
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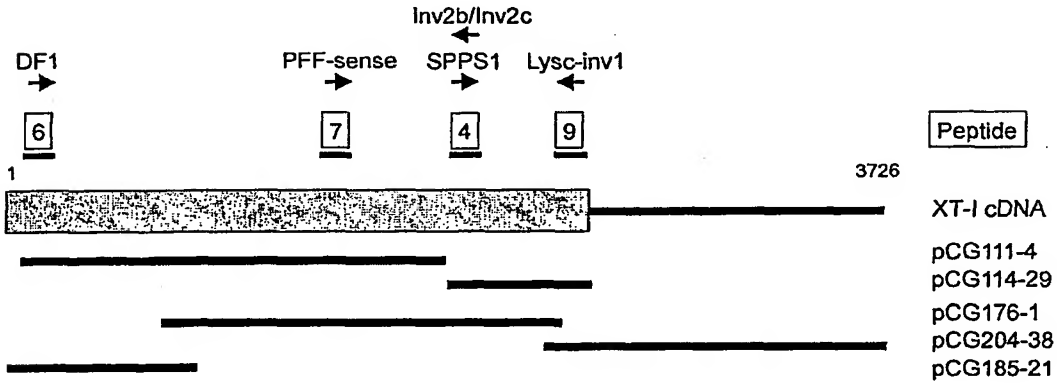

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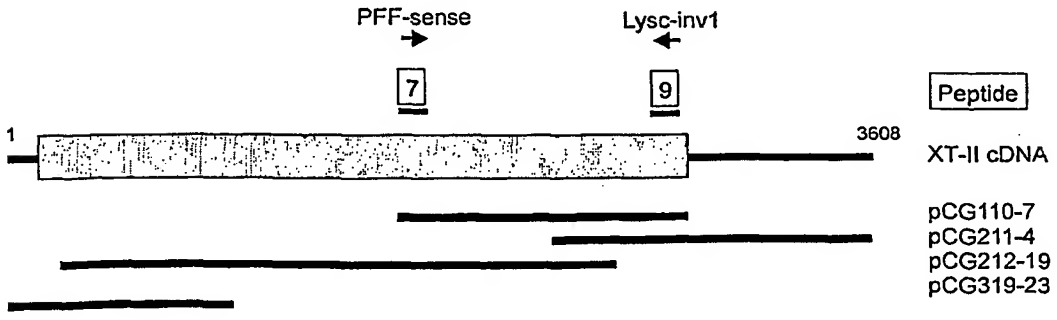

Fig. 7A

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TCTGTGCACCTCTACTTCCTTGCTGACCGCTTCCAGGGCTTTCTGATCAAGCATCATGCTACCAATCTG
GCTGTGAGCAAACTAGAGACTCTGGAGACCTGGGTGATGCCGAAAAAAGTCTTCAAGATCGCAAGCCCA
CCCAGTGACTTTGGGAGGCTTCAGTTTTCCGAGGTCGGCACTGACTGGGATGCCAAGGAGAGGCTATTC
CGCAACTTTGGGGGTCTTCTGGGGCCCATGGATGAGCCGGTGGGTATGCAGAAGTGGGGGAAGGGACCT
AATGTGACCGTGACCGTCATTTGGGTGGATCCCGTCAATGTCATCGCAGCCACCTACGACATCCTCATT
GAGTCCACTGCCGAATTCACACACTACAAGCCCCCTTTGAACTTGCCCCTGAGGCCTGGGGTCTGGACA
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GAGCAGAGCTTCCAGAGCCTAAACCCCGTCCTCAGCCTGCCCATCAACCCCGCCCAGGTGGAACAGGCA
CGGAGGAACGCAGCCTCCACGGGCACAGCGCTGGAGGGATGGCTGGACTCGTTGGTGGGCGGGATGTGG
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TGGAGCTCCTTCAGCCCTGACCCCAAGTCGGAGCTGGGTGCCGTCAAACCTGATGGCCGGCTCAGGTAG
CACTGGGCACGAGGAGTGGGCCACAGCAGGATCTCAACGGGAAAGCAGCCAGAGGGGTTGTGGGGCCTG
AACCCCGGCCTCCACCCTGGGGGAGGCCCTCTGTGAATGGGTCTCTCCTGGCCATAGAATGATGGAAAG
GGAAGGTCAGCAGGTCAAAGCAGGATCAGCCAACAACCTGCCTTTGGCAAGCTGCGGGTGGGATGGCTC
AGTCCCTGCACTGTGACTGTCTCACCTCTTCTGSTTGATCCTCAAGTCCTACAGGTTCCTTGTCTTCCC
CTTCCAGTGACCCACCCCTGACCCCAGACGTGTGATTTTCAGACTTTTCTTTCGAGCAGCAGAACTTCG
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TACCTCTTCAGAACCCAAGGGCTCTGCAAATGCCAGTTTGACAAGCACTGCCCAGACCAACCATGGGTT
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CCCTCCTTGGGTTGAACTATTCTCCTTAGTAACTAGGTAAGTGGGAAAGCCTTTTGATGTGGCATGGCC
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CAAAGTAGCAGAAATAGGATGATGTTTGTGAGCACCAGACTCAAGACCATGACCTTCTTTGATCCTTGA
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GTCAGACACACAAAACCTTCGTCAATTGCACACCGGTGCTGTTGGGAGTGACCAAACCACATGAACCAG
ACTTTTCCCGTCCAGGAAATAGCATTTCAGATTTGGTTTTTAATTTCATGCCCTTCGGCCACAGGCTCA
ACGGGACATGCAACATAAAAATGGGAAGGTTATTCAACCCGCGGGAAAAAAAAAAAAAAAAAAAAAAA

Fig. 7B

Thr Gln Asp Gly Tyr Phe Ser His Arg Pro Lys Glu Lys Val Arg Thr
Asp Ser Asn Asn Glu Asn Ser Val Pro Lys Asp Phe Glu Asn Val Asp
Asn Ser Asn Phe Ala Pro Arg Thr Gln Lys Gln Lys His Gln Pro Glu
Leu Ala Lys Lys Pro Pro Ser Arg Gln Lys Glu Leu Leu Lys Arg Lys
Leu Glu Gln Gln Glu Lys Gly Lys Gly His Thr Phe Pro Gly Lys Gly
Pro Gly Glu Val Leu Pro Pro Gly Asp Arg Ala Ala Ala Asn Ser Ser
His Gly Lys Asp Val Ser Arg Pro Pro His Ala Arg Lys Thr Gly Gly
Ser Ser Pro Glu Thr Lys Tyr Asp Gln Pro Pro Lys Cys Asp Ile Ser
Gly Lys Glu Ala Ile Ser Ala Leu Ser Arg Ala Lys Ser Lys His Cys
Arg Gln Glu Ile Gly Glu Thr Tyr Cys Arg His Lys Leu Gly Leu Leu
Met Pro Glu Lys Val Thr Arg Phe Cys Pro Leu Glu Gly Lys Ala Asn
Lys Asn Val Gln Trp **Asp Glu Asp** Ser Val Glu Tyr Met Pro Ala Asn
Pro Val Arg Ile Ala Phe Val Leu Val Val His Gly Arg Ala Ser Arg
Gln Leu Gln Arg Met Phe Lys Ala Ile Tyr His Lys Asp His Phe Tyr
Tyr Ile His Val Asp Lys Arg Ser Asn Tyr Leu His Arg Gln Val Leu
Gln Val Ser Arg Gln Tyr Ser Asn Val Arg Val Thr Pro Trp Arg Met
Ala Thr Ile Trp Gly Gly Ala Ser Leu Leu Ser Thr Tyr Leu Gln Ser
Met Arg Asp Leu Leu Glu Met Thr Asp Trp Pro Trp Asp Phe Phe Ile
Asn Leu Ser Ala Ala Asp Tyr Pro Ile Arg Thr Asn Asp Gln Leu Val
Ala Phe Leu Ser Arg Tyr Arg Asp Met Asn Phe Leu Lys Ser His Gly
Arg Asp Asn Ala Arg Phe Ile Arg Lys Gln Gly Leu Asp Arg Leu Phe
Leu Glu Cys Asp Ala His Met Trp Arg Leu Gly Asp Arg Arg Ile Pro
Glu Gly Ile Ala Val Asp Gly Gly Ser Asp Trp Phe Leu Leu Asn Arg
Arg Phe Val Glu Tyr Val Thr Phe Ser Thr Asp Asp Leu Val Thr Lys
Met Lys Gln Phe Tyr Ser Tyr Thr Leu Leu Pro Ala Glu Ser Phe Phe
His Thr Val Leu Glu Asn Ser Pro His Cys Asp Thr Met Val Asp Asn
Asn Leu Arg Ile Thr Asn Trp Asn Arg Lys Leu Gly Cys Lys Cys Gln
Tyr Lys His Ile Val Asp Trp Cys Gly Cys Ser Pro Asn Asp Phe Lys
Pro Gln Asp Phe His Arg Phe Gln Gln Thr Ala Arg Pro Thr Phe Phe
Ala Arg Lys Phe Glu Ala Val Val Asn Gln Glu Ile Ile Gly Gln Leu
Asp Tyr Tyr Leu Tyr Gly Asn Tyr Pro Ala Gly Thr Pro Gly Leu Arg
Ser Tyr Trp Glu Asn Val Tyr Asp Glu Pro Asp Gly Ile His Ser Leu
Ser Asp Val Thr Leu Thr Leu Tyr His Ser Phe Ala Arg Leu Gly Leu
Arg Arg Ala Glu Thr Ser Leu His Thr Asp Gly Glu Asn Ser Cys Arg
Tyr Tyr Pro Met Gly His Pro Ala Ser Val His Leu Tyr Phe Leu Ala
Asp Arg Phe Gln Gly Phe Leu Ile Lys His His Ala Thr Asn Leu Ala
Val Ser Lys Leu Glu Thr Leu Glu Thr Trp Val Met Pro Lys Lys Val
Phe Lys Ile Ala Ser Pro Pro Ser Asp Phe Gly Arg Leu Gln Phe Ser
Glu Val Gly Thr Asp Trp Asp Ala Lys Glu Arg Leu Phe Arg Asn Phe
Gly Gly Leu Leu Gly Pro Met Asp Glu Pro Val Gly Met Gln Lys Trp
Gly Lys Gly Pro Asn Val Thr Val Thr Val Ile Trp Val Asp Pro Val
Asn Val Ile Ala Ala Thr Tyr Asp Ile Leu Ile Glu Ser Thr Ala Glu
Phe Thr His Tyr Lys Pro Pro Leu Asn Leu Pro Leu Arg Pro Gly Val
Trp Thr Val Lys Ile Leu His His Trp Val Pro Val Ala Glu Thr Lys
Phe Leu Val Ala Pro Leu Thr Phe Ser Asn Arg Gln Pro Ile Lys Pro
Glu Glu Ala Leu Lys Leu His Asn Gly Pro Leu Arg Asn Ala Tyr Met
Glu Gln Ser Phe Gln Ser Leu Asn Pro Val Leu Ser Leu Pro Ile Asn
Pro Ala Gln Val Glu Gln Ala Arg Arg Asn Ala Ala Ser Thr Gly Thr
Ala Leu Glu Gly Trp Leu Asp Ser Leu Val Gly Gly Met Trp Thr Ala
Met Asp Ile Cys Ala Thr Gly Pro Thr Ala Cys Pro Val Met Gln Thr
Cys Ser Gln Thr Ala Trp Ser Ser Phe Ser Pro Asp Pro Lys Ser Glu
Leu Gly Ala Val Lys Pro Asp Gly Arg Leu Arg

Fig.8A

CGCAGGCCCCGCCCCCGGCCCCGCCCCGGCGCGCGCCCGGCCCGCCTCCCTAGGCGTGGAGGAGGGGGGG
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GGCAGGAAG**ATG**GTGGCGAGCGCGCGAGTGCAGAAGCTGGTGCGGCGCTACAAGCTGGCGATTGCCACGG
CGCTGGCCATCCTGCTGCTGCAGGGCCTGGTAGTGTGGAGCTTCAGCGGCCTGGAGGAGGACGAGGCGGG
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AGTGCGAGATCGTGGGCAAGGACGCACTGTCTGCACTGGCCCGGGCCAGCACCAAGCAGTGCCAGCAGGA
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CGGTGCGAATCGCCTACATGCTGGTGGTTCACGGCCGCGCCATCCGCCAGCTGAAGCGTCTCCTCAAGGC
CGTTTATCACGAGCAGCACTTCTTTTACATCCATGTGGACAAGCGTTCCGACTACCTGCACCGGGAGGTG
GTGGAGCTGGCCCAGGGCTATGATAACGTGCGGGTGACGCCCTGGCGCATGGTTACCATCTGGGGCGGGG
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TACACAGCCTTCGCCCGCCTCAGCCTGCACCATGCCGCCACTGCTGCACCCCCAATGGGCACCCCACTCT
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CCTGGTGACGCAGGCGGTGCAGCCCTCAGCCCAGGGGCCGGCAGAGACGCTTGAGATGTGGCTGATGCCC
CAAGGGTCGCTGAAGCTGTTGGGGCGCAGTGACCAGGCCAGCCGGCTCCAGAGTCTGGAGGTTGGCACTG
ATTGGGACCCCAAAGAGCGTCTTTTCCGGAACTTTGGGGGGTTACTGGGGCCGCTGGACGAGCCTGTGGC
CGTGCAGCGCTGGGCCCGGGGCCCCAACCTCACAGCCACAGTGGTCTGGATCGACCCAACCTATGTGGTG
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ACGGGCGACTC**AGG**TAGCAGGGCCCCAGCCAGTACCCGTGGAGGACCCGGGAAATTGCACCTTACAGACA
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CCCATTCTGGGCCTGTGGTGCTCGTGGCTGAGGCTCCACAGGGCTGCAAGTGCCCTGCCAGGCTCTAAGG
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GGACCCTAGAAGTGGGGTGGGGCGGCTCCAGGGCTTTCCAGCATACCCTGCCCCTGGATGGGAAAGGCAG
GGTCAGGGCCCACTGAGACCCATGCAGAGTTTCCAGGTGTGCACTGGAAGTGAGGTCACATGAGCAGCGT
GGGAAGAAGACTCTGTCAAGACTCTCAGAAGAACCTGGAGTAATTGTGCCTGAAGCTCAGCGTGAAGTCT
GAAATATGCAACAGAAGAAATATATCTCTATCTCTCTA

Fig. 8B

Met Val Ala Ser Ala Arg Val Gln Lys Leu Val Arg Arg Tyr Lys Leu Ala
Ile Ala Thr Ala Leu Ala Ile Leu Leu Leu Gln Gly Leu Val Val Trp Ser
Phe Ser Gly Leu Glu Glu Asp Glu Ala Gly Glu Lys Gly Arg Gln Arg Lys
Pro Arg Pro Leu Asp Pro Gly Glu Gly Ser Lys Asp Thr Asp Ser Ser Ala
Gly Arg Arg Gly Ser Thr Gly Arg Arg His Gly Arg Trp Arg Gly Arg Ala
Glu Ser Pro Gly Val Pro Val Ala Lys Val Val Arg Ala Val Thr Ser Arg
Gln Arg Ala Ser Arg Arg Val Pro Pro Ala Pro Pro Pro Glu Ala Pro Gly
Arg Gln Asn Leu Ser Gly Ala Ala Ala Gly Glu Ala Leu Val Gly Ala Ala
Gly Phe Pro Pro His Gly Asp Thr Gly Ser Val Glu Gly Ala Pro Gln Pro
Thr Asp Asn Gly Phe Thr Pro Lys Cys Glu Ile Val Gly Lys Asp Ala Leu
Ser Ala Leu Ala Arg Ala Ser Thr Lys Gln Cys Gln Gln Glu Ile Ala Asn
Val Val Cys Leu His Gln Ala Gly Ser Leu Met Pro Lys Ala Val Pro Arg
His Cys Gln Leu Thr Gly Lys Met Ser Pro Gly Ile Gln Trp Asp Glu Ser
Gln Ala Gln Gln Pro Met Asp Gly Pro Pro Val Arg Ile Ala Tyr Met Leu
Val Val His Gly Arg Ala Ile Arg Gln Leu Lys Arg Leu Leu Lys Ala Val
Tyr His Glu Gln His Phe Phe Tyr Ile His Val Asp Lys Arg Ser Asp Tyr
Leu His Arg Glu Val Val Glu Leu Ala Gln Gly Tyr Asp Asn Val Arg Val
Thr Pro Trp Arg Met Val Thr Ile Trp Gly Gly Ala Ser Leu Leu Thr Met
Tyr Leu Arg Ser Met Arg Asp Leu Leu Glu Val Pro Gly Trp Ala Trp Asp
Phe Phe Ile Asn Leu Ser Ala Thr Asp Tyr Pro Thr Arg Thr Asn Glu Glu
Leu Val Ala Phe Leu Ser Lys Asn Arg Asp Lys Asn Phe Leu Lys Ser His
Gly Arg Asp Asn Ser Arg Phe Ile Lys Lys Gln Gly Leu Asp Arg Leu Phe
His Glu Cys Asp Ser His Met Trp Arg Leu Gly Glu Arg Gln Ile Pro Ala
Gly Ile Val Val Asp Gly Gly Ser Asp Trp Phe Val Leu Thr Arg Ser Phe
Val Glu Tyr Val Val Tyr Thr Asp Asp Pro Leu Val Ala Gln Leu Arg Gln
Phe Tyr Thr Tyr Thr Leu Leu Pro Ala Glu Ser Phe Phe His Thr Val Leu
Glu Asn Ser Leu Ala Cys Glu Thr Leu Val Asp Asn Asn Leu Arg Val Thr
Asn Trp Asn Arg Lys Leu Gly Cys Lys Cys Gln Tyr Lys His Ile Val Asp
Trp Cys Gly Cys Ser Pro Asn Asp Phe Lys Pro Gln Asp Phe Leu Arg Leu
Gln Gln Val Ser Arg Pro Thr Phe Phe Ala Arg Lys Phe Glu Ser Thr Val
Asn Gln Glu Val Leu Glu Ile Leu Asp Phe His Leu Tyr Gly Ser Tyr Pro
Pro Gly Thr Pro Ala Leu Lys Ala Tyr Trp Glu Asn Thr Tyr Asp Ala Ala
Asp Gly Pro Ser Gly Leu Ser Asp Val Met Leu Thr Ala Tyr Thr Ala Phe
Ala Arg Leu Ser Leu His His Ala Ala Thr Ala Ala Pro Pro Met Gly Thr
Pro Leu Cys Arg Phe Glu Pro Arg Gly Leu Pro Ser Ser Val His Leu Tyr
Phe Tyr Asp Asp His Phe Gln Gly Tyr Leu Val Thr Gln Ala Val Gln Pro
Ser Ala Gln Gly Pro Ala Glu Thr Leu Glu Met Trp Leu Met Pro Gln Gly
Ser Leu Lys Leu Leu Gly Arg Ser Asp Gln Ala Ser Arg Leu Gln Ser Leu
Glu Val Gly Thr Asp Trp Asp Pro Lys Glu Arg Leu Phe Arg Asn Phe Gly
Gly Leu Leu Gly Pro Leu Asp Glu Pro Val Ala Val Gln Arg Trp Ala Arg
Gly Pro Asn Leu Thr Ala Thr Val Val Trp Ile Asp Pro Thr Tyr Val Val
Ala Thr Ser Tyr Asp Ile Thr Val Asp Thr Glu Thr Glu Val Thr Gln Tyr
Lys Pro Pro Leu Ser Arg Pro Leu Arg Pro Gly Pro Trp Thr Val Arg Leu
Leu Gln Phe Trp Glu Pro Leu Gly Glu Thr Arg Phe Leu Val Leu Pro Leu
Thr Phe Asn Arg Lys Leu Pro Leu Arg Lys Asp Asp Ala Ser Trp Leu His
Ala Gly Pro Pro His Asn Glu Tyr Met Glu Gln Ser Phe Gln Gly Leu Ser
Ser Ile Leu Asn Leu Pro Gln Pro Glu Leu Ala Glu Glu Ala Ala Gln Arg
His Thr Gln Leu Thr Gly Pro Ala Leu Glu Ala Trp Thr Asp Arg Glu Leu
Ser Ser Phe Trp Ser Val Ala Gly Leu Cys Ala Ile Gly Pro Ser Pro Cys
Pro Ser Leu Glu Pro Cys Arg Leu Thr Ser Trp Ser Ser Leu Ser Pro Asp
Pro Lys Ser Glu Leu Gly Pro Val Lys Ala Asp Gly Arg Leu Arg

Fig. 9

TACTTCTCTCACCGGCCCAAAGAGAAAGTTCGGACCGACAGCAACAATGAGAACTCAGTCCCCAA
GGACTTTGAGAATGTGGACAACAGCAACTTCGCACCCAGGACTCAAAAGCAGAAGCACCAGCCAG
AGTTGGCAAAGAAGCCCCTCAGCAGGCAGAAAGAGCGTTTGCAGAGAAAGCTGGGCGCCCAGGAC
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TCAGCGCTGACCCGCGCTAAGTCCAAGCACTGTCGCCAGGAGATTGCAGAAACCTACTGTCGCCA
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GGCCACATGGGACTGATACAGCCTGAACCCTGGCCTCTGATCTTATATGGGGGAGGGGTTCCTTC
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Fig. 10

GCGCTCGCGGCCGGGTTGCAGGGCCGGACGCGCGCCCCGCGTCCCGGGCAGGAAGATGGTGGCGA
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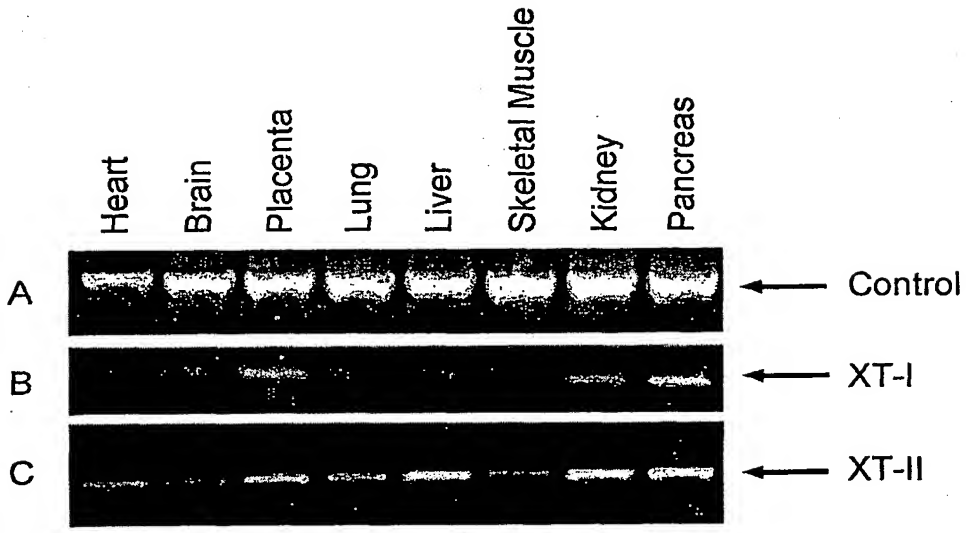
Fig. 11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Brain
Placenta
Lung
Liver
Skeletal Muscle
Kidney
Pancreas
A
B
C
Control
XT-I
XT-II

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Thr Gln Asp Gly Tyr Phe Ser His Arg Pro Lys Glu Lys Val Arg Thr
  1               5                  10                  15

gac agc aac aac gag aac tct gtc ccc aaa gac ttt gag aat gtg gac   96
Asp Ser Asn Asn Glu Asn Ser Val Pro Lys Asp Phe Glu Asn Val Asp
             20                  25                  30

aac agc aac ttc gca ccc agg act caa aag cag aag cac cag cct gag   144
Asn Ser Asn Phe Ala Pro Arg Thr Gln Lys Gln Lys His Gln Pro Glu
         35                  40                  45

ttg gcg aag aag cca ccg agt aga cag aag gag ctt ttg aaa agg aag   192
Leu Ala Lys Lys Pro Pro Ser Arg Gln Lys Glu Leu Leu Lys Arg Lys
     50                  55                  60
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ctg gaa cag cag gag aaa gga aaa gga cat aca ttc cct ggg aaa ggc   240
Leu Glu Gln Gln Glu Lys Gly Lys Gly His Thr Phe Pro Gly Lys Gly
 65                  70                  75                  80

ccc ggt gag gtg ctg cct ccc ggg gac aga gcc gca gcc aac agc agc   288
Pro Gly Glu Val Leu Pro Pro Gly Asp Arg Ala Ala Ala Asn Ser Ser
                 85                  90                  95

cac ggg aag gat gtg tcc aga ccg cct cat gcc agg aaa act ggg ggc   336
His Gly Lys Asp Val Ser Arg Pro Pro His Ala Arg Lys Thr Gly Gly
            100                 105                 110

agc tcc ccc gag acc aag tat gac cag ccc cct aag tgt gac atc tca   384
Ser Ser Pro Glu Thr Lys Tyr Asp Gln Pro Pro Lys Cys Asp Ile Ser
        115                 120                 125

ggc aag gag gcc atc tct gcc ctg tcc cgt gct aag tcc aag cac tgc   432
Gly Lys Glu Ala Ile Ser Ala Leu Ser Arg Ala Lys Ser Lys His Cys
    130                 135                 140

cgc cag gag att ggg gag act tac tgc cgc cac aag tta ggg ctg ctg   480
Arg Gln Glu Ile Gly Glu Thr Tyr Cys Arg His Lys Leu Gly Leu Leu
145                 150                 155                 160

atg cct gag aag gtg act cgg ttc tgc ccc ctc gag ggt aaa gcc aac   528
Met Pro Glu Lys Val Thr Arg Phe Cys Pro Leu Glu Gly Lys Ala Asn
                165                 170                 175

aag aac gtg cag tgg gac gag gac tcc gtg gag tac atg cca gcc aac   576
Lys Asn Val Gln Trp Asp Glu Asp Ser Val Glu Tyr Met Pro Ala Asn
            180                 185                 190

ccg gtc aga atc gcc ttt gtc ctg gtg gtc cac ggc cgt gcc tct cgg   624
Pro Val Arg Ile Ala Phe Val Leu Val Val His Gly Arg Ala Ser Arg
        195                 200                 205

cag ttg cag cgc atg ttc aag gcc atc tac cac aaa gac cac ttc tac   672
Gln Leu Gln Arg Met Phe Lys Ala Ile Tyr His Lys Asp His Phe Tyr
    210                 215                 220

tac atc cac gtg gac aag cgc tct aat tac ctg cat cgg caa gtg ctc   720
Tyr Ile His Val Asp Lys Arg Ser Asn Tyr Leu His Arg Gln Val Leu
225                 230                 235                 240

cag gtc tcc agg cag tac agc aat gtc cgc gtc acc ccc tgg aga atg   768
Gln Val Ser Arg Gln Tyr Ser Asn Val Arg Val Thr Pro Trp Arg Met
                245                 250                 255

gcc acc atc tgg gga gga gcc agc ctc ctg tcc acc tac ctg cag agc   816
Ala Thr Ile Trp Gly Gly Ala Ser Leu Leu Ser Thr Tyr Leu Gln Ser
            260                 265                 270

atg cgg gac ctc ctg gag atg acc gac tgg ccc tgg gac ttc ttc atc   864
Met Arg Asp Leu Leu Glu Met Thr Asp Trp Pro Trp Asp Phe Phe Ile
        275                 280                 285

aac ctg agt gcg gcc gac tac ccc atc agg aca aat gac cag ttg gtg   912
Asn Leu Ser Ala Ala Asp Tyr Pro Ile Arg Thr Asn Asp Gln Leu Val
    290                 295                 300

gcg ttt ctc tcc cga tac cga gat atg aat ttc ttg aag tca cac ggc   960
Ala Phe Leu Ser Arg Tyr Arg Asp Met Asn Phe Leu Lys Ser His Gly
305                 310                 315                 320
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cgg gac aat gca agg ttc att cgg aag cag ggc ctg gat cgg ctc ttc   1008
Arg Asp Asn Ala Arg Phe Ile Arg Lys Gln Gly Leu Asp Arg Leu Phe
                325                 330                 335

ctg gag tgc gac gct cac atg tgg cgc ctg gga gat cgg cgg atc cca   1056
Leu Glu Cys Asp Ala His Met Trp Arg Leu Gly Asp Arg Arg Ile Pro
            340                 345                 350

gag ggc att gcc gtg gat ggc ggt tcg gac tgg ttc ctg ctg aac cgg   1104
Glu Gly Ile Ala Val Asp Gly Gly Ser Asp Trp Phe Leu Leu Asn Arg
        355                 360                 365

agg ttt gta gaa tat gtg acc ttc tcc aca gac gat ctg gtg acc aag   1152
Arg Phe Val Glu Tyr Val Thr Phe Ser Thr Asp Asp Leu Val Thr Lys
    370                 375                 380

atg aaa cag ttc tac tcc tac acc ctg ctt cct gct gag tcc ttc ttc   1200
Met Lys Gln Phe Tyr Ser Tyr Thr Leu Leu Pro Ala Glu Ser Phe Phe
385                 390                 395                 400

cat acg gtc ctg gag aac agc ccc cac tgc gac acc atg gtg gac aac   1248
His Thr Val Leu Glu Asn Ser Pro His Cys Asp Thr Met Val Asp Asn
                405                 410                 415

aac ctg cgc atc acc aac tgg aat cgc aag ctg ggc tgc aag tgc cag   1296
Asn Leu Arg Ile Thr Asn Trp Asn Arg Lys Leu Gly Cys Lys Cys Gln
            420                 425                 430

tac aag cac atc gtg gac tgg tgc ggc tgc tcc ccc aat gac ttc aag   1344
Tyr Lys His Ile Val Asp Trp Cys Gly Cys Ser Pro Asn Asp Phe Lys
        435                 440                 445

ccg cag gac ttc cac cgc ttc cag cag aca gcc cgg cct acc ttc ttt   1392
Pro Gln Asp Phe His Arg Phe Gln Gln Thr Ala Arg Pro Thr Phe Phe
    450                 455                 460

gcc cgc aag ttt gaa gcc gtg gtg aat cag gaa atc att ggg cag ctg   1440
Ala Arg Lys Phe Glu Ala Val Val Asn Gln Glu Ile Ile Gly Gln Leu
465                 470                 475                 480

gac tat tac ctg tac ggg aac tac cct gca ggt acc ccg ggc ctg cgc   1488
Asp Tyr Tyr Leu Tyr Gly Asn Tyr Pro Ala Gly Thr Pro Gly Leu Arg
                485                 490                 495

tcc tac tgg gag aat gtc tac gat gag cct gac ggc atc cac agc ctg   1536
Ser Tyr Trp Glu Asn Val Tyr Asp Glu Pro Asp Gly Ile His Ser Leu
            500                 505                 510

agc gac gtg aca ctc acc ttg tac cac tcc ttt gcc cgc ctg ggt ctt   1584
Ser Asp Val Thr Leu Thr Leu Tyr His Ser Phe Ala Arg Leu Gly Leu
        515                 520                 525

cga cgg gct gag acg tcc ctg cac acg gat ggg gag aac agc tgc cga   1632
Arg Arg Ala Glu Thr Ser Leu His Thr Asp Gly Glu Asn Ser Cys Arg
    530                 535                 540

tac tac cca atg ggc cac cca gca tct gtg cac ctc tac ttc ctt gct   1680
Tyr Tyr Pro Met Gly His Pro Ala Ser Val His Leu Tyr Phe Leu Ala
545                 550                 555                 560

gac cgc ttc cag ggc ttt ctg atc aag cat cat gct acc aat ctg gct   1728
Asp Arg Phe Gln Gly Phe Leu Ile Lys His His Ala Thr Asn Leu Ala
                565                 570                 575
```

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gtg agc aaa cta gag act ctg gag acc tgg gtg atg ccg aaa aaa gtc   1776
Val Ser Lys Leu Glu Thr Leu Glu Thr Trp Val Met Pro Lys Lys Val
            580                 585                 590

ttc aag atc gca agc cca ccc agt gac ttt ggg agg ctt cag ttt tcc   1824
Phe Lys Ile Ala Ser Pro Pro Ser Asp Phe Gly Arg Leu Gln Phe Ser
        595                 600                 605

gag gtc ggc act gac tgg gat gcc aag gag agg cta ttc cgc aac ttt   1872
Glu Val Gly Thr Asp Trp Asp Ala Lys Glu Arg Leu Phe Arg Asn Phe
    610                 615                 620

ggg ggt ctt ctg ggg ccc atg gat gag ccg gtg ggt atg cag aag tgg   1920
Gly Gly Leu Leu Gly Pro Met Asp Glu Pro Val Gly Met Gln Lys Trp
625                 630                 635                 640

ggg aag gga cct aat gtg acc gtg acc gtc att tgg gtg gat ccc gtc   1968
Gly Lys Gly Pro Asn Val Thr Val Thr Val Ile Trp Val Asp Pro Val
                645                 650                 655

aat gtc atc gca gcc acc tac gac atc ctc att gag tcc act gcc gaa   2016
Asn Val Ile Ala Ala Thr Tyr Asp Ile Leu Ile Glu Ser Thr Ala Glu
            660                 665                 670

ttc aca cac tac aag ccc cct ttg aac ttg ccc ctg agg cct ggg gtc   2064
Phe Thr His Tyr Lys Pro Pro Leu Asn Leu Pro Leu Arg Pro Gly Val
        675                 680                 685

tgg aca gtg aaa att ctc cac cac tgg gtg cca gtt gca gag acc aaa   2112
Trp Thr Val Lys Ile Leu His His Trp Val Pro Val Ala Glu Thr Lys
    690                 695                 700

ttc ctc gtt gcg cct ctg acc ttc tcg aac agg cag ccc atc aaa cct   2160
Phe Leu Val Ala Pro Leu Thr Phe Ser Asn Arg Gln Pro Ile Lys Pro
705                 710                 715                 720

gag gag gca ctg aag ctg cac aat ggg ccc ctc cgc aat gcc tac atg   2208
Glu Glu Ala Leu Lys Leu His Asn Gly Pro Leu Arg Asn Ala Tyr Met
                725                 730                 735

gag cag agc ttc cag agc cta aac ccc gtc ctc agc ctg ccc atc aac   2256
Glu Gln Ser Phe Gln Ser Leu Asn Pro Val Leu Ser Leu Pro Ile Asn
            740                 745                 750

ccc gcc cag gtg gaa cag gca cgg agg aac gca gcc tcc acg ggc aca   2304
Pro Ala Gln Val Glu Gln Ala Arg Arg Asn Ala Ala Ser Thr Gly Thr
        755                 760                 765

gcg ctg gag gga tgg ctg gac tcg ttg gtg ggc ggg atg tgg act gcc   2352
Ala Leu Glu Gly Trp Leu Asp Ser Leu Val Gly Gly Met Trp Thr Ala
    770                 775                 780

atg gac atc tgt gcc acg ggc ccc aca gcc tgc ccg gtc atg cag acc   2400
Met Asp Ile Cys Ala Thr Gly Pro Thr Ala Cys Pro Val Met Gln Thr
785                 790                 795                 800

tgc agc cag acg gcc tgg agc tcc ttc agc cct gac ccc aag tcg gag   2448
Cys Ser Gln Thr Ala Trp Ser Ser Phe Ser Pro Asp Pro Lys Ser Glu
                805                 810                 815

ctg ggt gcc gtc aaa cct gat ggc cgg ctc agg tag cactgggcac        2494
Leu Gly Ala Val Lys Pro Asp Gly Arg Leu Arg
            820                 825
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Asn Ser Asn Phe Ala Pro Arg Thr Gln Lys Gln Lys His Gln Pro Glu
35 40 45

Leu Ala Lys Lys Pro Pro Ser Arg Gln Lys Glu Leu Leu Lys Arg Lys
50 55 60

Leu Glu Gln Gln Glu Lys Gly Lys Gly His Thr Phe Pro Gly Lys Gly
65 70 75 80

Pro Gly Glu Val Leu Pro Pro Gly Asp Arg Ala Ala Ala Asn Ser Ser
85 90 95

His Gly Lys Asp Val Ser Arg Pro Pro His Ala Arg Lys Thr Gly Gly
100 105 110

Ser Ser Pro Glu Thr Lys Tyr Asp Gln Pro Pro Lys Cys Asp Ile Ser
115 120 125

Gly Lys Glu Ala Ile Ser Ala Leu Ser Arg Ala Lys Ser Lys His Cys
130 135 140

Arg Gln Glu Ile Gly Glu Thr Tyr Cys Arg His Lys Leu Gly Leu Leu
145 150 155 160

Met Pro Glu Lys Val Thr Arg Phe Cys Pro Leu Glu Gly Lys Ala Asn
165 170 175

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Lys Asn Val Gln Trp Asp Glu Asp Ser Val Glu Tyr Met Pro Ala Asn
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Pro Val Arg Ile Ala Phe Val Leu Val Val His Gly Arg Ala Ser Arg
        195                 200                 205

Gln Leu Gln Arg Met Phe Lys Ala Ile Tyr His Lys Asp His Phe Tyr
    210                 215                 220

Tyr Ile His Val Asp Lys Arg Ser Asn Tyr Leu His Arg Gln Val Leu
225                 230                 235                 240

Gln Val Ser Arg Gln Tyr Ser Asn Val Arg Val Thr Pro Trp Arg Met
                245                 250                 255

Ala Thr Ile Trp Gly Gly Ala Ser Leu Leu Ser Thr Tyr Leu Gln Ser
            260                 265                 270

Met Arg Asp Leu Leu Glu Met Thr Asp Trp Pro Trp Asp Phe Phe Ile
        275                 280                 285

Asn Leu Ser Ala Ala Asp Tyr Pro Ile Arg Thr Asn Asp Gln Leu Val
    290                 295                 300

Ala Phe Leu Ser Arg Tyr Arg Asp Met Asn Phe Leu Lys Ser His Gly
305                 310                 315                 320

Arg Asp Asn Ala Arg Phe Ile Arg Lys Gln Gly Leu Asp Arg Leu Phe
                325                 330                 335

Leu Glu Cys Asp Ala His Met Trp Arg Leu Gly Asp Arg Arg Ile Pro
            340                 345                 350

Glu Gly Ile Ala Val Asp Gly Gly Ser Asp Trp Phe Leu Leu Asn Arg
        355                 360                 365

Arg Phe Val Glu Tyr Val Thr Phe Ser Thr Asp Asp Leu Val Thr Lys
    370                 375                 380

Met Lys Gln Phe Tyr Ser Tyr Thr Leu Leu Pro Ala Glu Ser Phe Phe
385                 390                 395                 400

His Thr Val Leu Glu Asn Ser Pro His Cys Asp Thr Met Val Asp Asn
                405                 410                 415

Asn Leu Arg Ile Thr Asn Trp Asn Arg Lys Leu Gly Cys Lys Cys Gln
            420                 425                 430

Tyr Lys His Ile Val Asp Trp Cys Gly Cys Ser Pro Asn Asp Phe Lys
        435                 440                 445

Pro Gln Asp Phe His Arg Phe Gln Gln Thr Ala Arg Pro Thr Phe Phe
    450                 455                 460

Ala Arg Lys Phe Glu Ala Val Val Asn Gln Glu Ile Ile Gly Gln Leu
465                 470                 475                 480

Asp Tyr Tyr Leu Tyr Gly Asn Tyr Pro Ala Gly Thr Pro Gly Leu Arg
                485                 490                 495

Ser Tyr Trp Glu Asn Val Tyr Asp Glu Pro Asp Gly Ile His Ser Leu
            500                 505                 510
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Ser Asp Val Thr Leu Thr Leu Tyr His Ser Phe Ala Arg Leu Gly Leu
        515                 520                 525

Arg Arg Ala Glu Thr Ser Leu His Thr Asp Gly Glu Asn Ser Cys Arg
    530                 535                 540

Tyr Tyr Pro Met Gly His Pro Ala Ser Val His Leu Tyr Phe Leu Ala
545                 550                 555                 560

Asp Arg Phe Gln Gly Phe Leu Ile Lys His His Ala Thr Asn Leu Ala
                565                 570                 575

Val Ser Lys Leu Glu Thr Leu Glu Thr Trp Val Met Pro Lys Lys Val
            580                 585                 590

Phe Lys Ile Ala Ser Pro Pro Ser Asp Phe Gly Arg Leu Gln Phe Ser
        595                 600                 605

Glu Val Gly Thr Asp Trp Asp Ala Lys Glu Arg Leu Phe Arg Asn Phe
    610                 615                 620

Gly Gly Leu Leu Gly Pro Met Asp Glu Pro Val Gly Met Gln Lys Trp
625                 630                 635                 640

Gly Lys Gly Pro Asn Val Thr Val Thr Val Ile Trp Val Asp Pro Val
                645                 650                 655

Asn Val Ile Ala Ala Thr Tyr Asp Ile Leu Ile Glu Ser Thr Ala Glu
            660                 665                 670

Phe Thr His Tyr Lys Pro Pro Leu Asn Leu Pro Leu Arg Pro Gly Val
        675                 680                 685

Trp Thr Val Lys Ile Leu His His Trp Val Pro Val Ala Glu Thr Lys
    690                 695                 700

Phe Leu Val Ala Pro Leu Thr Phe Ser Asn Arg Gln Pro Ile Lys Pro
705                 710                 715                 720

Glu Glu Ala Leu Lys Leu His Asn Gly Pro Leu Arg Asn Ala Tyr Met
                725                 730                 735

Glu Gln Ser Phe Gln Ser Leu Asn Pro Val Leu Ser Leu Pro Ile Asn
            740                 745                 750

Pro Ala Gln Val Glu Gln Ala Arg Arg Asn Ala Ala Ser Thr Gly Thr
        755                 760                 765

Ala Leu Glu Gly Trp Leu Asp Ser Leu Val Gly Gly Met Trp Thr Ala
    770                 775                 780

Met Asp Ile Cys Ala Thr Gly Pro Thr Ala Cys Pro Val Met Gln Thr
785                 790                 795                 800

Cys Ser Gln Thr Ala Trp Ser Ser Phe Ser Pro Asp Pro Lys Ser Glu
                805                 810                 815

Leu Gly Ala Val Lys Pro Asp Gly Arg Leu Arg
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Met Val Ala Ser Ala Arg Val Gln
1 5

aag ctg gtg cgg cgc tac aag ctg gcg att gcc acg gcg ctg gcc atc 221
Lys Leu Val Arg Arg Tyr Lys Leu Ala Ile Ala Thr Ala Leu Ala Ile
10 15 20

ctg ctg ctg cag ggc ctg gta gtg tgg agc ttc agc ggc ctg gag gag 269
Leu Leu Leu Gln Gly Leu Val Val Trp Ser Phe Ser Gly Leu Glu Glu
25 30 35 40

gac gag gcg ggc gag aaa gga agg cag agg aag cca cgg cca ctg gac 317
Asp Glu Ala Gly Glu Lys Gly Arg Gln Arg Lys Pro Arg Pro Leu Asp
45 50 55

cct ggc gaa ggt tcc aag gac aca gac agt tca gca ggg cga cgg ggc 365
Pro Gly Glu Gly Ser Lys Asp Thr Asp Ser Ser Ala Gly Arg Arg Gly
60 65 70

agc aca ggc aga agg cat ggg cgc tgg cgg ggc cgt gct gag agc cca 413
Ser Thr Gly Arg Arg His Gly Arg Trp Arg Gly Arg Ala Glu Ser Pro
75 80 85

gga gtg ccc gtg gcc aag gtg gta cgg gca gta acc agc cgg cag aga 461
Gly Val Pro Val Ala Lys Val Val Arg Ala Val Thr Ser Arg Gln Arg
90 95 100

gcc agc cgg cgg gtc cca cct gcc cca cct ccg gaa gcc cca ggc cgc 509
Ala Ser Arg Arg Val Pro Pro Ala Pro Pro Pro Glu Ala Pro Gly Arg
105 110 115 120

cag aac ctg agt ggg gca gca gct ggg gag gcg ctg gta ggg gca gct 557
Gln Asn Leu Ser Gly Ala Ala Ala Gly Glu Ala Leu Val Gly Ala Ala
125 130 135

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ggc ttc cca cca cac gga gat aca ggg agc gtg gag ggc gcc ccc cag   605
Gly Phe Pro Pro His Gly Asp Thr Gly Ser Val Glu Gly Ala Pro Gln
            140                 145                 150

ccc acg gac aat ggc ttc acc ccc aag tgc gag atc gtg ggc aag gac   653
Pro Thr Asp Asn Gly Phe Thr Pro Lys Cys Glu Ile Val Gly Lys Asp
        155                 160                 165

gca ctg tct gca ctg gcc cgg gcc agc acc aag cag tgc cag cag gag   701
Ala Leu Ser Ala Leu Ala Arg Ala Ser Thr Lys Gln Cys Gln Gln Glu
    170                 175                 180

atc gcc aat gtg gtg tgc ctg cac cag gct ggg agc ctc atg ccc aag   749
Ile Ala Asn Val Val Cys Leu His Gln Ala Gly Ser Leu Met Pro Lys
185                 190                 195                 200

gct gtg ccc cgg cac tgt cag ctg act ggg aag atg agc ccc ggc atc   797
Ala Val Pro Arg His Cys Gln Leu Thr Gly Lys Met Ser Pro Gly Ile
                205                 210                 215

cag tgg gat gag agc caa gcc cag cag ccc atg gat ggc ccc ccg gtg   845
Gln Trp Asp Glu Ser Gln Ala Gln Gln Pro Met Asp Gly Pro Pro Val
            220                 225                 230

cga atc gcc tac atg ctg gtg gtt cac ggc cgc gcc atc cgc cag ctg   893
Arg Ile Ala Tyr Met Leu Val Val His Gly Arg Ala Ile Arg Gln Leu
        235                 240                 245

aag cgt ctc ctc aag gcc gtt tat cac gag cag cac ttc ttt tac atc   941
Lys Arg Leu Leu Lys Ala Val Tyr His Glu Gln His Phe Phe Tyr Ile
    250                 255                 260

cat gtg gac aag cgt tcc gac tac ctg cac cgg gag gtg gtg gag ctg   989
His Val Asp Lys Arg Ser Asp Tyr Leu His Arg Glu Val Val Glu Leu
265                 270                 275                 280

gcc cag ggc tat gat aac gtg cgg gtg acg ccc tgg cgc atg gtt acc   1037
Ala Gln Gly Tyr Asp Asn Val Arg Val Thr Pro Trp Arg Met Val Thr
                285                 290                 295

atc tgg ggc ggg gcc agc ctc ctg acg atg tac ctg cgg agc atg cgg   1085
Ile Trp Gly Gly Ala Ser Leu Leu Thr Met Tyr Leu Arg Ser Met Arg
            300                 305                 310

gac ctg cta gag gtg cct ggc tgg gcc tgg gac ttc ttc atc aac ctc   1133
Asp Leu Leu Glu Val Pro Gly Trp Ala Trp Asp Phe Phe Ile Asn Leu
        315                 320                 325

agt gcc act gac tat cca acc agg acc aat gag gag ctg gtg gca ttc   1181
Ser Ala Thr Asp Tyr Pro Thr Arg Thr Asn Glu Glu Leu Val Ala Phe
    330                 335                 340

cta tcc aag aac cgg gac aag aat ttc ctc aag tca cat ggc cgg gac   1229
Leu Ser Lys Asn Arg Asp Lys Asn Phe Leu Lys Ser His Gly Arg Asp
345                 350                 355                 360

aac tcc agg ttc atc aag aaa cag ggc ctg gac cgg ctc ttc cat gag   1277
Asn Ser Arg Phe Ile Lys Lys Gln Gly Leu Asp Arg Leu Phe His Glu
                365                 370                 375

tgc gac tca cac atg tgg cgc ctg ggc gag cgg cag atc cca gca ggc   1325
Cys Asp Ser His Met Trp Arg Leu Gly Glu Arg Gln Ile Pro Ala Gly
            380                 385                 390
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att gtg gtg gat ggc ggt tct gac tgg ttc gtg ctg aca cgc agc ttt   1373
Ile Val Val Asp Gly Gly Ser Asp Trp Phe Val Leu Thr Arg Ser Phe
        395                 400                 405

gtg gag tat gtg gtg tac aca gat gac ccg ctt gtg gcc cag ctg cgc   1421
Val Glu Tyr Val Val Tyr Thr Asp Asp Pro Leu Val Ala Gln Leu Arg
    410                 415                 420

cag ttc tac aca tac aca ctg ctc cca gcc gag tcc ttc ttc cac acg   1469
Gln Phe Tyr Thr Tyr Thr Leu Leu Pro Ala Glu Ser Phe Phe His Thr
425                 430                 435                 440

gtg ctg gag aac agc ctg gcc tgt gag acc ctc gtg gac aac aac ctg   1517
Val Leu Glu Asn Ser Leu Ala Cys Glu Thr Leu Val Asp Asn Asn Leu
                445                 450                 455

cgg gtc acc aac tgg aac cgc aag ctg ggc tgc aag tgc cag tac aag   1565
Arg Val Thr Asn Trp Asn Arg Lys Leu Gly Cys Lys Cys Gln Tyr Lys
            460                 465                 470

cac att gtg gac tgg tgt ggc tgc tcc ccc aac gac ttc aag cca cag   1613
His Ile Val Asp Trp Cys Gly Cys Ser Pro Asn Asp Phe Lys Pro Gln
        475                 480                 485

gac ttc ctc cgg ctg cag caa gtc tcc aga ccc acc ttc ttc gcc cgg   1661
Asp Phe Leu Arg Leu Gln Gln Val Ser Arg Pro Thr Phe Phe Ala Arg
    490                 495                 500

aag ttc gag tcg act gtg aac cag gag gtg ctg gaa atc ctg gac ttc   1709
Lys Phe Glu Ser Thr Val Asn Gln Glu Val Leu Glu Ile Leu Asp Phe
505                 510                 515                 520

cac ctg tac ggc agc tac ccc ccc ggc acg cca gcc ctc aag gcc tac   1757
His Leu Tyr Gly Ser Tyr Pro Pro Gly Thr Pro Ala Leu Lys Ala Tyr
                525                 530                 535

tgg gag aac acc tac gac gcg gct gat ggc ccc agt ggg ctc agt gat   1805
Trp Glu Asn Thr Tyr Asp Ala Ala Asp Gly Pro Ser Gly Leu Ser Asp
            540                 545                 550

gtc atg ctc act gct tac aca gcc ttc gcc cgc ctc agc ctg cac cat   1853
Val Met Leu Thr Ala Tyr Thr Ala Phe Ala Arg Leu Ser Leu His His
        555                 560                 565

gcc gcc act gct gca ccc cca atg ggc acc cca ctc tgc agg ttt gag   1901
Ala Ala Thr Ala Ala Pro Pro Met Gly Thr Pro Leu Cys Arg Phe Glu
    570                 575                 580

ccc agg ggc ttg ccg tcc agc gtg cac ctg tat ttc tat gac gac cat   1949
Pro Arg Gly Leu Pro Ser Ser Val His Leu Tyr Phe Tyr Asp Asp His
585                 590                 595                 600

ttc cag ggc tac ctg gtg acg cag gcg gtg cag ccc tca gcc cag ggg   1997
Phe Gln Gly Tyr Leu Val Thr Gln Ala Val Gln Pro Ser Ala Gln Gly
                605                 610                 615

ccg gca gag acg ctt gag atg tgg ctg atg ccc caa ggg tcg ctg aag   2045
Pro Ala Glu Thr Leu Glu Met Trp Leu Met Pro Gln Gly Ser Leu Lys
            620                 625                 630

ctg ttg ggg cgc agt gac cag gcc agc cgg ctc cag agt ctg gag gtt   2093
Leu Leu Gly Arg Ser Asp Gln Ala Ser Arg Leu Gln Ser Leu Glu Val
        635                 640                 645
```

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ggc act gat tgg gac ccc aaa gag cgt ctt ttc cgg aac ttt ggg ggg   2141
Gly Thr Asp Trp Asp Pro Lys Glu Arg Leu Phe Arg Asn Phe Gly Gly
    650                 655                 660

tta ctg ggg ccg ctg gac gag cct gtg gcc gtg cag cgc tgg gcc cgg   2189
Leu Leu Gly Pro Leu Asp Glu Pro Val Ala Val Gln Arg Trp Ala Arg
665                 670                 675                 680

ggc ccc aac ctc aca gcc aca gtg gtc tgg atc gac cca acc tat gtg   2237
Gly Pro Asn Leu Thr Ala Thr Val Val Trp Ile Asp Pro Thr Tyr Val
                685                 690                 695

gtg gcc aca tct tat gac atc aca gta gat acg gag act gag gtc acg   2285
Val Ala Thr Ser Tyr Asp Ile Thr Val Asp Thr Glu Thr Glu Val Thr
            700                 705                 710

caa tac aag ccc cca ctg agc cgg ccc ctg cgg cca ggg ccc tgg act   2333
Gln Tyr Lys Pro Pro Leu Ser Arg Pro Leu Arg Pro Gly Pro Trp Thr
        715                 720                 725

gtt cga ctc ctt cag ttc tgg gaa ccg ctg ggt gag acc cgc ttc ctt   2381
Val Arg Leu Leu Gln Phe Trp Glu Pro Leu Gly Glu Thr Arg Phe Leu
    730                 735                 740

gtg ctg ccc ttg acc ttc aac cgc aaa cta cct ctc agg aaa gat gat   2429
Val Leu Pro Leu Thr Phe Asn Arg Lys Leu Pro Leu Arg Lys Asp Asp
745                 750                 755                 760

gcc agc tgg ctg cac gca ggg cca ccc cac aac gag tac atg gag cag   2477
Ala Ser Trp Leu His Ala Gly Pro Pro His Asn Glu Tyr Met Glu Gln
                765                 770                 775

agt ttc cag ggc ctg agt agc atc ctg aac ctg cct cag ccg gag ctc   2525
Ser Phe Gln Gly Leu Ser Ser Ile Leu Asn Leu Pro Gln Pro Glu Leu
            780                 785                 790

gcg gag gag gct gcc cag cgg cac aca cag ctc aca ggc cct gcg ctc   2573
Ala Glu Glu Ala Ala Gln Arg His Thr Gln Leu Thr Gly Pro Ala Leu
        795                 800                 805

gag gcc tgg aca gac agg gaa ctg agc agc ttc tgg tcc gtg gct gga   2621
Glu Ala Trp Thr Asp Arg Glu Leu Ser Ser Phe Trp Ser Val Ala Gly
    810                 815                 820

ctg tgt gcc ata ggc ccc tct ccc tgc ccc tcc ctg gag ccc tgc aga   2669
Leu Cys Ala Ile Gly Pro Ser Pro Cys Pro Ser Leu Glu Pro Cys Arg
825                 830                 835                 840

ctg acc agc tgg agc tct ctg tcc ccc gac ccc aaa tca gag ctg ggg   2717
Leu Thr Ser Trp Ser Ser Leu Ser Pro Asp Pro Lys Ser Glu Leu Gly
                845                 850                 855

cct gtc aaa gca gac ggg cga ctc agg tagcagggcc ccagccagta         2764
Pro Val Lys Ala Asp Gly Arg Leu Arg
            860                 865

cccgtggagg acccgggaaa ttgcacctta cagacagtgg aggggtgtcc cctcccacag 2824
gcaagaacca gaggcccagg ctgcacaccc atttcagcca tcaagaaccc acacagacgg 2884
cagggaaggt ggacacagta tgaactactg ctgatgtctc tgttggggat cagagggctg 2944
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aagtgggcca tgtctgtggg gagcagtggc ttgctgagcc cacctgctat tgttctgcac 3184
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gaggctgggc ctgcctcact ctccagggcc tcatgcccca ttctgggcct gtggtgctcg 3244
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tcta 3608

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Ala Ile Ala Thr Ala Leu Ala Ile Leu Leu Leu Gln Gly Leu Val Val
20 25 30

Trp Ser Phe Ser Gly Leu Glu Glu Asp Glu Ala Gly Glu Lys Gly Arg
35 40 45

Gln Arg Lys Pro Arg Pro Leu Asp Pro Gly Glu Gly Ser Lys Asp Thr
50 55 60

Asp Ser Ser Ala Gly Arg Arg Gly Ser Thr Gly Arg Arg His Gly Arg
65 70 75 80

Trp Arg Gly Arg Ala Glu Ser Pro Gly Val Pro Val Ala Lys Val Val
85 90 95

Arg Ala Val Thr Ser Arg Gln Arg Ala Ser Arg Arg Val Pro Pro Ala
100 105 110

Pro Pro Pro Glu Ala Pro Gly Arg Gln Asn Leu Ser Gly Ala Ala Ala
115 120 125

Gly Glu Ala Leu Val Gly Ala Ala Gly Phe Pro Pro His Gly Asp Thr
130 135 140

Gly Ser Val Glu Gly Ala Pro Gln Pro Thr Asp Asn Gly Phe Thr Pro
145 150 155 160

Lys Cys Glu Ile Val Gly Lys Asp Ala Leu Ser Ala Leu Ala Arg Ala
165 170 175

Ser Thr Lys Gln Cys Gln Gln Glu Ile Ala Asn Val Val Cys Leu His
180 185 190

Gln Ala Gly Ser Leu Met Pro Lys Ala Val Pro Arg His Cys Gln Leu
195 200 205

Thr Gly Lys Met Ser Pro Gly Ile Gln Trp Asp Glu Ser Gln Ala Gln
210 215 220

Gln Pro Met Asp Gly Pro Pro Val Arg Ile Ala Tyr Met Leu Val Val
225 230 235 240

His Gly Arg Ala Ile Arg Gln Leu Lys Arg Leu Leu Lys Ala Val Tyr
245 250 255

```
His Glu Gln His Phe Phe Tyr Ile His Val Asp Lys Arg Ser Asp Tyr
            260                 265                 270

Leu His Arg Glu Val Val Glu Leu Ala Gln Gly Tyr Asp Asn Val Arg
        275                 280                 285

Val Thr Pro Trp Arg Met Val Thr Ile Trp Gly Gly Ala Ser Leu Leu
    290                 295                 300

Thr Met Tyr Leu Arg Ser Met Arg Asp Leu Leu Glu Val Pro Gly Trp
305                 310                 315                 320

Ala Trp Asp Phe Phe Ile Asn Leu Ser Ala Thr Asp Tyr Pro Thr Arg
                325                 330                 335

Thr Asn Glu Glu Leu Val Ala Phe Leu Ser Lys Asn Arg Asp Lys Asn
            340                 345                 350

Phe Leu Lys Ser His Gly Arg Asp Asn Ser Arg Phe Ile Lys Lys Gln
        355                 360                 365

Gly Leu Asp Arg Leu Phe His Glu Cys Asp Ser His Met Trp Arg Leu
    370                 375                 380

Gly Glu Arg Gln Ile Pro Ala Gly Ile Val Val Asp Gly Gly Ser Asp
385                 390                 395                 400

Trp Phe Val Leu Thr Arg Ser Phe Val Glu Tyr Val Val Tyr Thr Asp
                405                 410                 415

Asp Pro Leu Val Ala Gln Leu Arg Gln Phe Tyr Thr Tyr Thr Leu Leu
            420                 425                 430

Pro Ala Glu Ser Phe Phe His Thr Val Leu Glu Asn Ser Leu Ala Cys
        435                 440                 445

Glu Thr Leu Val Asp Asn Asn Leu Arg Val Thr Asn Trp Asn Arg Lys
    450                 455                 460

Leu Gly Cys Lys Cys Gln Tyr Lys His Ile Val Asp Trp Cys Gly Cys
465                 470                 475                 480

Ser Pro Asn Asp Phe Lys Pro Gln Asp Phe Leu Arg Leu Gln Gln Val
                485                 490                 495

Ser Arg Pro Thr Phe Phe Ala Arg Lys Phe Glu Ser Thr Val Asn Gln
            500                 505                 510

Glu Val Leu Glu Ile Leu Asp Phe His Leu Tyr Gly Ser Tyr Pro Pro
        515                 520                 525

Gly Thr Pro Ala Leu Lys Ala Tyr Trp Glu Asn Thr Tyr Asp Ala Ala
    530                 535                 540

Asp Gly Pro Ser Gly Leu Ser Asp Val Met Leu Thr Ala Tyr Thr Ala
545                 550                 555                 560

Phe Ala Arg Leu Ser Leu His His Ala Ala Thr Ala Ala Pro Pro Met
                565                 570                 575

Gly Thr Pro Leu Cys Arg Phe Glu Pro Arg Gly Leu Pro Ser Ser Val
            580                 585                 590
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```
His Leu Tyr Phe Tyr Asp Asp His Phe Gln Gly Tyr Leu Val Thr Gln
        595                 600                 605

Ala Val Gln Pro Ser Ala Gln Gly Pro Ala Glu Thr Leu Glu Met Trp
    610                 615                 620

Leu Met Pro Gln Gly Ser Leu Lys Leu Leu Gly Arg Ser Asp Gln Ala
625                 630                 635                 640

Ser Arg Leu Gln Ser Leu Glu Val Gly Thr Asp Trp Asp Pro Lys Glu
                645                 650                 655

Arg Leu Phe Arg Asn Phe Gly Gly Leu Leu Gly Pro Leu Asp Glu Pro
            660                 665                 670

Val Ala Val Gln Arg Trp Ala Arg Gly Pro Asn Leu Thr Ala Thr Val
        675                 680                 685

Val Trp Ile Asp Pro Thr Tyr Val Val Ala Thr Ser Tyr Asp Ile Thr
    690                 695                 700

Val Asp Thr Glu Thr Glu Val Thr Gln Tyr Lys Pro Pro Leu Ser Arg
705                 710                 715                 720

Pro Leu Arg Pro Gly Pro Trp Thr Val Arg Leu Leu Gln Phe Trp Glu
                725                 730                 735

Pro Leu Gly Glu Thr Arg Phe Leu Val Leu Pro Leu Thr Phe Asn Arg
            740                 745                 750

Lys Leu Pro Leu Arg Lys Asp Asp Ala Ser Trp Leu His Ala Gly Pro
        755                 760                 765

Pro His Asn Glu Tyr Met Glu Gln Ser Phe Gln Gly Leu Ser Ser Ile
    770                 775                 780

Leu Asn Leu Pro Gln Pro Glu Leu Ala Glu Glu Ala Ala Gln Arg His
785                 790                 795                 800

Thr Gln Leu Thr Gly Pro Ala Leu Glu Ala Trp Thr Asp Arg Glu Leu
                805                 810                 815

Ser Ser Phe Trp Ser Val Ala Gly Leu Cys Ala Ile Gly Pro Ser Pro
            820                 825                 830

Cys Pro Ser Leu Glu Pro Cys Arg Leu Thr Ser Trp Ser Ser Leu Ser
        835                 840                 845

Pro Asp Pro Lys Ser Glu Leu Gly Pro Val Lys Ala Asp Gly Arg Leu
    850                 855                 860

Arg
865
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Tyr Phe Ser His Arg Pro Lys Glu Lys Val Arg Thr Asp Ser Asn Asn
 1               5                  10                  15

gag aac tca gtc ccc aag gac ttt gag aat gtg gac aac agc aac ttc   96
Glu Asn Ser Val Pro Lys Asp Phe Glu Asn Val Asp Asn Ser Asn Phe
            20                  25                  30

gca ccc agg act caa aag cag aag cac cag cca gag ttg gca aag aag   144
Ala Pro Arg Thr Gln Lys Gln Lys His Gln Pro Glu Leu Ala Lys Lys
        35                  40                  45

ccc ctc agc agg cag aaa gag cgt ttg cag aga aag ctg ggc gcc cag   192
Pro Leu Ser Arg Gln Lys Glu Arg Leu Gln Arg Lys Leu Gly Ala Gln
    50                  55                  60

gac aaa gga cag ggg cag tca gtc cta gga aaa ggc ccc aag gag gtg   240
Asp Lys Gly Gln Gly Gln Ser Val Leu Gly Lys Gly Pro Lys Glu Val
65                  70                  75                  80

ctg cct cct cgg gaa aaa gcc cca ggc aac agt agc caa ggg aag gat   288
Leu Pro Pro Arg Glu Lys Ala Pro Gly Asn Ser Ser Gln Gly Lys Asp
                85                  90                  95

ctc tca aga cac agc cat agc agg aag agt ggt ggc ggt ggg tcc ccg   336
Leu Ser Arg His Ser His Ser Arg Lys Ser Gly Gly Gly Gly Ser Pro
            100                 105                 110

gaa act aag tct gac cag gtc ccc aag tgt gac atc tct ggc aag gag   384
Glu Thr Lys Ser Asp Gln Val Pro Lys Cys Asp Ile Ser Gly Lys Glu
        115                 120                 125

gcc atc tca gcg ctg acc cgc gct aag tcc aag cac tgt cgc cag gag   432
Ala Ile Ser Ala Leu Thr Arg Ala Lys Ser Lys His Cys Arg Gln Glu
    130                 135                 140

att gca gaa acc tac tgt cgc cac aag ctg ggg ctg ctg atg cca gag   480
Ile Ala Glu Thr Tyr Cys Arg His Lys Leu Gly Leu Leu Met Pro Glu
145                 150                 155                 160

aag gtg gct cga ttc tgt ccc ctg gaa ggc aaa gcc aac aag aat gtc   528
Lys Val Ala Arg Phe Cys Pro Leu Glu Gly Lys Ala Asn Lys Asn Val
                165                 170                 175

cag tgg gat gag gat gct gtt gag tac atg ccc ccc aac cca gtc aga   576
Gln Trp Asp Glu Asp Ala Val Glu Tyr Met Pro Pro Asn Pro Val Arg
            180                 185                 190

atc gcc ttt gtc ctg gtg gtc cat ggc cgt gcc tct cga cag ctg cag   624
Ile Ala Phe Val Leu Val Val His Gly Arg Ala Ser Arg Gln Leu Gln
        195                 200                 205

cgc atg ttt aag gcc atc tac cac aaa gac cat ttc tac tat atc cat   672
Arg Met Phe Lys Ala Ile Tyr His Lys Asp His Phe Tyr Tyr Ile His
    210                 215                 220

gtg gat aag cgt tcc aat tac ctg cat cgg caa gtg ctc cag ttc tcc   720
Val Asp Lys Arg Ser Asn Tyr Leu His Arg Gln Val Leu Gln Phe Ser
225                 230                 235                 240

agg cag tac gac aac gtc cga gtc act tcc tgg agg atg gcc acc att   768
Arg Gln Tyr Asp Asn Val Arg Val Thr Ser Trp Arg Met Ala Thr Ile
                245                 250                 255
```

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tgg ggc gga gcc agc ctc ctg tcc act tac ctg cag agc atg cgg gat   816
Trp Gly Gly Ala Ser Leu Leu Ser Thr Tyr Leu Gln Ser Met Arg Asp
            260                 265                 270

cta ctg gag atg act gac tgg ccc tgg gac ttc ttc atc aac ctg agt   864
Leu Leu Glu Met Thr Asp Trp Pro Trp Asp Phe Phe Ile Asn Leu Ser
        275                 280                 285

gcg gct gac tac ccc atc agg aca aat gac cag ctc gta gca ttt ctt   912
Ala Ala Asp Tyr Pro Ile Arg Thr Asn Asp Gln Leu Val Ala Phe Leu
    290                 295                 300

tcc aga tac cga gat atg aat ttc ctg aag tca cat ggt cgg gac aat   960
Ser Arg Tyr Arg Asp Met Asn Phe Leu Lys Ser His Gly Arg Asp Asn
305                 310                 315                 320

gca agg ttc atc cgg aag cag gac ctg gac cgt ctc ttc ctg gag tgt   1008
Ala Arg Phe Ile Arg Lys Gln Asp Leu Asp Arg Leu Phe Leu Glu Cys
                325                 330                 335

gat aca cac atg tgg cgc ctg ggg gac cgg cga atc cca gag ggc att   1056
Asp Thr His Met Trp Arg Leu Gly Asp Arg Arg Ile Pro Glu Gly Ile
            340                 345                 350

gct gta gat ggc ggc tct gat tgg ttc ctg cta aac cgg aag ttt gta   1104
Ala Val Asp Gly Gly Ser Asp Trp Phe Leu Leu Asn Arg Lys Phe Val
        355                 360                 365

gag tat gtg gca ttc tcc aca gat gac ctg gtg acc aag atg aag cag   1152
Glu Tyr Val Ala Phe Ser Thr Asp Asp Leu Val Thr Lys Met Lys Gln
    370                 375                 380

ttc tac tct tat acc ctc ctc cct gct gag tcc ttt ttc cac acg gtc   1200
Phe Tyr Ser Tyr Thr Leu Leu Pro Ala Glu Ser Phe Phe His Thr Val
385                 390                 395                 400

ctg gag aac agc ccc cac tgt gac acc atg gtg gat aac aac ctg cgc   1248
Leu Glu Asn Ser Pro His Cys Asp Thr Met Val Asp Asn Asn Leu Arg
                405                 410                 415

atc acc aat tgg aac cgc aag ctg ggc tgc aag tgc cag tac aag cat   1296
Ile Thr Asn Trp Asn Arg Lys Leu Gly Cys Lys Cys Gln Tyr Lys His
            420                 425                 430

att gtg gac tgg tgt ggc tgc tct ccc aat gac ttc aag cct cag gac   1344
Ile Val Asp Trp Cys Gly Cys Ser Pro Asn Asp Phe Lys Pro Gln Asp
        435                 440                 445

ttc cat cgc ttc cag cag acg gcc cgg ccc acc ttc ttt gcc cga aag   1392
Phe His Arg Phe Gln Gln Thr Ala Arg Pro Thr Phe Phe Ala Arg Lys
    450                 455                 460

ttc gaa gcc ata gtg aac cag gaa atc att ggg cag ttg gac tct tac   1440
Phe Glu Ala Ile Val Asn Gln Glu Ile Ile Gly Gln Leu Asp Ser Tyr
465                 470                 475                 480

ctg tat ggc aac tat cct gcg ggc acc ccg ggc ctc cgc tct tac tgg   1488
Leu Tyr Gly Asn Tyr Pro Ala Gly Thr Pro Gly Leu Arg Ser Tyr Trp
                485                 490                 495

gag aat gtc tat gac gaa cca gat ggc atc cag agc ctc agc gat gtg   1536
Glu Asn Val Tyr Asp Glu Pro Asp Gly Ile Gln Ser Leu Ser Asp Val
            500                 505                 510
```

```
gca ctc acc atg tat cat tcc ttc atc cgc ctg ggt ctt cga agg gct   1584
Ala Leu Thr Met Tyr His Ser Phe Ile Arg Leu Gly Leu Arg Arg Ala
        515                 520                 525

gag tca tcg cta cac acg gat ggg gag aac agc tgc agg tac tat cca   1632
Glu Ser Ser Leu His Thr Asp Gly Glu Asn Ser Cys Arg Tyr Tyr Pro
    530                 535                 540

atg ggc cac cca gcg tct gtc cac ctc tac ttc ctg gct gac cga ttc   1680
Met Gly His Pro Ala Ser Val His Leu Tyr Phe Leu Ala Asp Arg Phe
545                 550                 555                 560

cag ggc ttt ctg atc aag cac cat gtg acc aac ctt gct gtg agc aaa   1728
Gln Gly Phe Leu Ile Lys His His Val Thr Asn Leu Ala Val Ser Lys
                565                 570                 575

ctg gag aca ctg gag aca tgg atg atg cca aag aaa gtc ttc aag gtc   1776
Leu Glu Thr Leu Glu Thr Trp Met Met Pro Lys Lys Val Phe Lys Val
            580                 585                 590

gca agt ccc ccc agt gac ttt ggg agg ctt cag ttt tct gag gtt ggc   1824
Ala Ser Pro Pro Ser Asp Phe Gly Arg Leu Gln Phe Ser Glu Val Gly
        595                 600                 605

act gac tgg gat gcc aag gag aga cta ttc cgg aac ttt ggt ggt ctt   1872
Thr Asp Trp Asp Ala Lys Glu Arg Leu Phe Arg Asn Phe Gly Gly Leu
    610                 615                 620

ttg ggg ccc atg gat gag ccg gtg gga atg cag aaa tgg ggg aag gga   1920
Leu Gly Pro Met Asp Glu Pro Val Gly Met Gln Lys Trp Gly Lys Gly
625                 630                 635                 640

ccc aat gtg acc gtg act gtt att tgg gtg gat cct gtc aat gtc atc   1968
Pro Asn Val Thr Val Thr Val Ile Trp Val Asp Pro Val Asn Val Ile
                645                 650                 655

gca gcc acc tat gat atc ctg atc gag tcc act gcg gaa ttc aca cac   2016
Ala Ala Thr Tyr Asp Ile Leu Ile Glu Ser Thr Ala Glu Phe Thr His
            660                 665                 670

tac aaa ccc cct ttg aat ctg cct ctg agg cct ggt gtc tgg aca gtg   2064
Tyr Lys Pro Pro Leu Asn Leu Pro Leu Arg Pro Gly Val Trp Thr Val
        675                 680                 685

aag att ctc cat cac tgg gta cca gtt gca gag acc aaa ttc ctt gtc   2112
Lys Ile Leu His His Trp Val Pro Val Ala Glu Thr Lys Phe Leu Val
    690                 695                 700

gca cct ctg acc ttc tcc aac aag cag ccc atc aaa cca gag gag gct   2160
Ala Pro Leu Thr Phe Ser Asn Lys Gln Pro Ile Lys Pro Glu Glu Ala
705                 710                 715                 720

ttg aag ctg cac aat ggg cca cct cgc agt gcc tac atg gag cag agt   2208
Leu Lys Leu His Asn Gly Pro Pro Arg Ser Ala Tyr Met Glu Gln Ser
                725                 730                 735

ttc cag agc cta aac cca gtc ctc agc ctg cac atc aat cct gcc caa   2256
Phe Gln Ser Leu Asn Pro Val Leu Ser Leu His Ile Asn Pro Ala Gln
            740                 745                 750

gtg gag cag gcc cgg aag aac gca gcc ttc act ggg aca gca cta gaa   2304
Val Glu Gln Ala Arg Lys Asn Ala Ala Phe Thr Gly Thr Ala Leu Glu
        755                 760                 765
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gcc tgg ctg gtg ggt ggg act tgg act gcc atg gac gtc tgt gcc aca   2352
Ala Trp Leu Val Gly Gly Thr Trp Thr Ala Met Asp Val Cys Ala Thr
    770                 775                 780

ggc ccc act gcc tgc cca gtc atg caa acc tgc agc caa aca gcc tgg   2400
Gly Pro Thr Ala Cys Pro Val Met Gln Thr Cys Ser Gln Thr Ala Trp
785                 790                 795                 800

agt tcc ttc agc cct gat ccc aag tca gag ctg ggt gca gtg aag cct   2448
Ser Ser Phe Ser Pro Asp Pro Lys Ser Glu Leu Gly Ala Val Lys Pro
                805                 810                 815

gat ggt cgg ctc agg                                             2463
Asp Gly Arg Leu Arg
            820
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Tyr Phe Ser His Arg Pro Lys Glu Lys Val Arg Thr Asp Ser Asn Asn
  1               5                  10                  15

Glu Asn Ser Val Pro Lys Asp Phe Glu Asn Val Asp Asn Ser Asn Phe
             20                  25                  30

Ala Pro Arg Thr Gln Lys Gln Lys His Gln Pro Glu Leu Ala Lys Lys
         35                  40                  45

Pro Leu Ser Arg Gln Lys Glu Arg Leu Gln Arg Lys Leu Gly Ala Gln
     50                  55                  60

Asp Lys Gly Gln Gly Gln Ser Val Leu Gly Lys Gly Pro Lys Glu Val
 65                  70                  75                  80

Leu Pro Pro Arg Glu Lys Ala Pro Gly Asn Ser Ser Gln Gly Lys Asp
                 85                  90                  95

Leu Ser Arg His Ser His Ser Arg Lys Ser Gly Gly Gly Gly Ser Pro
            100                 105                 110

Glu Thr Lys Ser Asp Gln Val Pro Lys Cys Asp Ile Ser Gly Lys Glu
        115                 120                 125

Ala Ile Ser Ala Leu Thr Arg Ala Lys Ser Lys His Cys Arg Gln Glu
    130                 135                 140

Ile Ala Glu Thr Tyr Cys Arg His Lys Leu Gly Leu Leu Met Pro Glu
145                 150                 155                 160

Lys Val Ala Arg Phe Cys Pro Leu Glu Gly Lys Ala Asn Lys Asn Val
                165                 170                 175

Gln Trp Asp Glu Asp Ala Val Glu Tyr Met Pro Pro Asn Pro Val Arg
            180                 185                 190

Ile Ala Phe Val Leu Val Val His Gly Arg Ala Ser Arg Gln Leu Gln
        195                 200                 205
```

```
Arg Met Phe Lys Ala Ile Tyr His Lys Asp His Phe Tyr Tyr Ile His
    210                 215                 220

Val Asp Lys Arg Ser Asn Tyr Leu His Arg Gln Val Leu Gln Phe Ser
225                 230                 235                 240

Arg Gln Tyr Asp Asn Val Arg Val Thr Ser Trp Arg Met Ala Thr Ile
                245                 250                 255

Trp Gly Gly Ala Ser Leu Leu Ser Thr Tyr Leu Gln Ser Met Arg Asp
            260                 265                 270

Leu Leu Glu Met Thr Asp Trp Pro Trp Asp Phe Phe Ile Asn Leu Ser
        275                 280                 285

Ala Ala Asp Tyr Pro Ile Arg Thr Asn Asp Gln Leu Val Ala Phe Leu
    290                 295                 300

Ser Arg Tyr Arg Asp Met Asn Phe Leu Lys Ser His Gly Arg Asp Asn
305                 310                 315                 320

Ala Arg Phe Ile Arg Lys Gln Asp Leu Asp Arg Leu Phe Leu Glu Cys
                325                 330                 335

Asp Thr His Met Trp Arg Leu Gly Asp Arg Arg Ile Pro Glu Gly Ile
            340                 345                 350

Ala Val Asp Gly Gly Ser Asp Trp Phe Leu Leu Asn Arg Lys Phe Val
        355                 360                 365

Glu Tyr Val Ala Phe Ser Thr Asp Asp Leu Val Thr Lys Met Lys Gln
    370                 375                 380

Phe Tyr Ser Tyr Thr Leu Leu Pro Ala Glu Ser Phe Phe His Thr Val
385                 390                 395                 400

Leu Glu Asn Ser Pro His Cys Asp Thr Met Val Asp Asn Asn Leu Arg
                405                 410                 415

Ile Thr Asn Trp Asn Arg Lys Leu Gly Cys Lys Cys Gln Tyr Lys His
            420                 425                 430

Ile Val Asp Trp Cys Gly Cys Ser Pro Asn Asp Phe Lys Pro Gln Asp
        435                 440                 445

Phe His Arg Phe Gln Gln Thr Ala Arg Pro Thr Phe Phe Ala Arg Lys
    450                 455                 460

Phe Glu Ala Ile Val Asn Gln Glu Ile Ile Gly Gln Leu Asp Ser Tyr
465                 470                 475                 480

Leu Tyr Gly Asn Tyr Pro Ala Gly Thr Pro Gly Leu Arg Ser Tyr Trp
                485                 490                 495

Glu Asn Val Tyr Asp Glu Pro Asp Gly Ile Gln Ser Leu Ser Asp Val
            500                 505                 510

Ala Leu Thr Met Tyr His Ser Phe Ile Arg Leu Gly Leu Arg Arg Ala
        515                 520                 525

Glu Ser Ser Leu His Thr Asp Gly Glu Asn Ser Cys Arg Tyr Tyr Pro
    530                 535                 540
```

```
Met Gly His Pro Ala Ser Val His Leu Tyr Phe Leu Ala Asp Arg Phe
545                 550                 555                 560

Gln Gly Phe Leu Ile Lys His His Val Thr Asn Leu Ala Val Ser Lys
                565                 570                 575

Leu Glu Thr Leu Glu Thr Trp Met Met Pro Lys Lys Val Phe Lys Val
            580                 585                 590

Ala Ser Pro Pro Ser Asp Phe Gly Arg Leu Gln Phe Ser Glu Val Gly
        595                 600                 605

Thr Asp Trp Asp Ala Lys Glu Arg Leu Phe Arg Asn Phe Gly Gly Leu
    610                 615                 620

Leu Gly Pro Met Asp Glu Pro Val Gly Met Gln Lys Trp Gly Lys Gly
625                 630                 635                 640

Pro Asn Val Thr Val Thr Val Ile Trp Val Asp Pro Val Asn Val Ile
                645                 650                 655

Ala Ala Thr Tyr Asp Ile Leu Ile Glu Ser Thr Ala Glu Phe Thr His
            660                 665                 670

Tyr Lys Pro Pro Leu Asn Leu Pro Leu Arg Pro Gly Val Trp Thr Val
        675                 680                 685

Lys Ile Leu His His Trp Val Pro Val Ala Glu Thr Lys Phe Leu Val
    690                 695                 700

Ala Pro Leu Thr Phe Ser Asn Lys Gln Pro Ile Lys Pro Glu Glu Ala
705                 710                 715                 720

Leu Lys Leu His Asn Gly Pro Pro Arg Ser Ala Tyr Met Glu Gln Ser
                725                 730                 735

Phe Gln Ser Leu Asn Pro Val Leu Ser Leu His Ile Asn Pro Ala Gln
            740                 745                 750

Val Glu Gln Ala Arg Lys Asn Ala Ala Phe Thr Gly Thr Ala Leu Glu
        755                 760                 765

Ala Trp Leu Val Gly Gly Thr Trp Thr Ala Met Asp Val Cys Ala Thr
    770                 775                 780

Gly Pro Thr Ala Cys Pro Val Met Gln Thr Cys Ser Gln Thr Ala Trp
785                 790                 795                 800

Ser Ser Phe Ser Pro Asp Pro Lys Ser Glu Leu Gly Ala Val Lys Pro
                805                 810                 815

Asp Gly Arg Leu Arg
            820
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<210> 7
<211> 2858
<212> DNA
<213> Rat coronavirus
<220>
<221> CDS
<222> (56)..(2650)
<223> rat XT-2

<400> 7

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gcgctcgcgg ccgggttgca gggccggacg cgcgccccgc gtcccgggca ggaag atg  58
                                                            Met
                                                              1

gtg gcg agc gcg cgg gtg cag aag ctg gtg cgg cgc tac aag ctg gcg  106
Val Ala Ser Ala Arg Val Gln Lys Leu Val Arg Arg Tyr Lys Leu Ala
              5                  10                  15

atc gcc acc gcg ctg gcc atc ctg ctg ctg cag ggc ctg gtg gtg tgg  154
Ile Ala Thr Ala Leu Ala Ile Leu Leu Leu Gln Gly Leu Val Val Trp
         20                  25                  30

agc ttc agc ggc ctg gag gag gac gag ccg ggc gag aaa gga agg cag  202
Ser Phe Ser Gly Leu Glu Glu Asp Glu Pro Gly Glu Lys Gly Arg Gln
     35                  40                  45

aga aag cca cgg ccg cta gac cct gga gag ggt tcc aag gac acg gac  250
Arg Lys Pro Arg Pro Leu Asp Pro Gly Glu Gly Ser Lys Asp Thr Asp
 50                  55                  60                  65

agc tct gca ggg cgt agg ggc agt gct ggg aga agg cac gga cgg tgg  298
Ser Ser Ala Gly Arg Arg Gly Ser Ala Gly Arg Arg His Gly Arg Trp
                 70                  75                  80

cgg ggc cgc gcc gag agc cca ggt gtg cct gtg gcc aag gtg gtg cgg  346
Arg Gly Arg Ala Glu Ser Pro Gly Val Pro Val Ala Lys Val Val Arg
             85                  90                  95

gca gta acc agt cgg cag aga gcc agc cga cgg gtg ccc cct gcc cca  394
Ala Val Thr Ser Arg Gln Arg Ala Ser Arg Arg Val Pro Pro Ala Pro
        100                 105                 110

cct cca gag gca cca ggc cgc cag aac ctg agt gga gca gca gcc ggg  442
Pro Pro Glu Ala Pro Gly Arg Gln Asn Leu Ser Gly Ala Ala Ala Gly
    115                 120                 125

gaa gca ctg ata ggt gcc gcc ggc ttc cca caa cat gga gac aca ggg  490
Glu Ala Leu Ile Gly Ala Ala Gly Phe Pro Gln His Gly Asp Thr Gly
130                 135                 140                 145

agt gtg gag ggt gcc cct cag ccc acg gat aac agc ttc act ccg aag  538
Ser Val Glu Gly Ala Pro Gln Pro Thr Asp Asn Ser Phe Thr Pro Lys
                150                 155                 160

tgt gag att gtg ggc aag gat gca ctg tca gca ctg gcc cgg gcc agc  586
Cys Glu Ile Val Gly Lys Asp Ala Leu Ser Ala Leu Ala Arg Ala Ser
            165                 170                 175

acc aag cat tgt caa caa gag att gct aat gta gtg tgc ctg cac caa  634
Thr Lys His Cys Gln Gln Glu Ile Ala Asn Val Val Cys Leu His Gln
        180                 185                 190

gct ggg aac cta atg ccc aag tct gtg ccc cgg cac tgc cag ctg gct  682
Ala Gly Asn Leu Met Pro Lys Ser Val Pro Arg His Cys Gln Leu Ala
    195                 200                 205

ggc aag gtg agc cct ggc atc cag tgg gaa gag gtc cgg gcc cag cag  730
Gly Lys Val Ser Pro Gly Ile Gln Trp Glu Glu Val Arg Ala Gln Gln
210                 215                 220                 225

cct gtg agt ggc cct ctg gta cgc atc gcc tac atg ctg gtg gtt cac  778
Pro Val Ser Gly Pro Leu Val Arg Ile Ala Tyr Met Leu Val Val His
                230                 235                 240
```

```
ggc cgt gct gtg cgc cag ctg aag cgt ctt ctg aag gcc gtc tac cac   826
Gly Arg Ala Val Arg Gln Leu Lys Arg Leu Leu Lys Ala Val Tyr His
            245                 250                 255

gag gag cac ttc ttt tat att cat gtg gac aag cgt tcc aac tac ctg   874
Glu Glu His Phe Phe Tyr Ile His Val Asp Lys Arg Ser Asn Tyr Leu
        260                 265                 270

tac cgg gag gtg gta gag ctg gcc cag cac tac gac aat gta cgg gta   922
Tyr Arg Glu Val Val Glu Leu Ala Gln His Tyr Asp Asn Val Arg Val
    275                 280                 285

aca cct tgg cgc atg gtc acc atc tgg ggt ggg gct agc ctt ctg agg   970
Thr Pro Trp Arg Met Val Thr Ile Trp Gly Gly Ala Ser Leu Leu Arg
290                 295                 300                 305

atg tac ctg cgg agc atg aag gac ctt ctg gag act cct ggc tgg acc   1018
Met Tyr Leu Arg Ser Met Lys Asp Leu Leu Glu Thr Pro Gly Trp Thr
                310                 315                 320

tgg gac ttc ttc atc aac ctg agc gct act gac tat cca acc agg acg   1066
Trp Asp Phe Phe Ile Asn Leu Ser Ala Thr Asp Tyr Pro Thr Arg Thr
            325                 330                 335

aat gag gag ctg gta gcg ttc tta tcc aag aac cgg gac aag aat ttc   1114
Asn Glu Glu Leu Val Ala Phe Leu Ser Lys Asn Arg Asp Lys Asn Phe
        340                 345                 350

ctc aag tca cac ggg cga gac aat tcc agg ttc atc aag aaa caa ggc   1162
Leu Lys Ser His Gly Arg Asp Asn Ser Arg Phe Ile Lys Lys Gln Gly
    355                 360                 365

ctg gac cgg ctt ttc cat gag tgt gat tcc cac atg tgg cgc ctg ggt   1210
Leu Asp Arg Leu Phe His Glu Cys Asp Ser His Met Trp Arg Leu Gly
370                 375                 380                 385

gaa cgg cag atc ccg gca ggc atc gtg gtg gat ggt ggc tct gac tgg   1258
Glu Arg Gln Ile Pro Ala Gly Ile Val Val Asp Gly Gly Ser Asp Trp
                390                 395                 400

ttc gtg ctg aca cgc agc ttt gtg gaa tat gtg gtg tat aca gag gat   1306
Phe Val Leu Thr Arg Ser Phe Val Glu Tyr Val Val Tyr Thr Glu Asp
            405                 410                 415

cct ctg gtg gcc cag ctt cgc cag ttc tat aca tac aca ttg ctt cca   1354
Pro Leu Val Ala Gln Leu Arg Gln Phe Tyr Thr Tyr Thr Leu Leu Pro
        420                 425                 430

gcc gag tcc ttc ttc cac aca gtg ctg gag aac agc cca gcc tgt gag   1402
Ala Glu Ser Phe Phe His Thr Val Leu Glu Asn Ser Pro Ala Cys Glu
    435                 440                 445

agc cta gtg gac aac aac ctg cgg gtt acc aac tgg aac cgg aag ctg   1450
Ser Leu Val Asp Asn Asn Leu Arg Val Thr Asn Trp Asn Arg Lys Leu
450                 455                 460                 465

ggc tgc aag tgc cag tac aag cac atc gtg gac tgg tgt ggc tgc tcc   1498
Gly Cys Lys Cys Gln Tyr Lys His Ile Val Asp Trp Cys Gly Cys Ser
                470                 475                 480

ccc aac gac ttc aag cca cag gac ttc ctg cgg ctt cag caa gtc tcc   1546
Pro Asn Asp Phe Lys Pro Gln Asp Phe Leu Arg Leu Gln Gln Val Ser
            485                 490                 495
```

```
aga ccc acc ttc ttt gcc cgg aag ttt gag tcg act gtg aac cag gaa   1594
Arg Pro Thr Phe Phe Ala Arg Lys Phe Glu Ser Thr Val Asn Gln Glu
        500                 505                 510

gtc ctg gaa att ttg gac ttc cac ctg tat ggc agc tac cca ccc ggc   1642
Val Leu Glu Ile Leu Asp Phe His Leu Tyr Gly Ser Tyr Pro Pro Gly
    515                 520                 525

acc cca gcc ctc aag gcc tac tgg gag aac atc tac gac atg gcc gat   1690
Thr Pro Ala Leu Lys Ala Tyr Trp Glu Asn Ile Tyr Asp Met Ala Asp
530                 535                 540                 545

ggc cct agt gga ctc agc gat gtc cta ctc act gct tac aca gcc ttt   1738
Gly Pro Ser Gly Leu Ser Asp Val Leu Leu Thr Ala Tyr Thr Ala Phe
                550                 555                 560

gcc cgt atc agt ctg cgt cat gct gcc act gtt tcc cca ctg gcc act   1786
Ala Arg Ile Ser Leu Arg His Ala Ala Thr Val Ser Pro Leu Ala Thr
            565                 570                 575

gca gtc tgc agg ttt gag ccc agg ggg ttg ccg tcc agc gtg cac ctg   1834
Ala Val Cys Arg Phe Glu Pro Arg Gly Leu Pro Ser Ser Val His Leu
        580                 585                 590

tat ttc tat gac gac cat ttc cag ggc tac ctg gtg acg cag gca gtg   1882
Tyr Phe Tyr Asp Asp His Phe Gln Gly Tyr Leu Val Thr Gln Ala Val
    595                 600                 605

cag ccc tca gcc cag ggg cca gca gag aca ctt gag atg tgg ctg atg   1930
Gln Pro Ser Ala Gln Gly Pro Ala Glu Thr Leu Glu Met Trp Leu Met
610                 615                 620                 625

ccc cag agg ttg ctg aag ccg ttg ggg cac agt gac cag gcc agc cgg   1978
Pro Gln Arg Leu Leu Lys Pro Leu Gly His Ser Asp Gln Ala Ser Arg
                630                 635                 640

ctc cag agt ctg gag gtt ggc act gag tgg gac ccc aaa gaa cgt ctc   2026
Leu Gln Ser Leu Glu Val Gly Thr Glu Trp Asp Pro Lys Glu Arg Leu
            645                 650                 655

ttc cgg aac ttt ggg ggc ctg ttg gga cca ctg gat gaa cct gtg gcc   2074
Phe Arg Asn Phe Gly Gly Leu Leu Gly Pro Leu Asp Glu Pro Val Ala
        660                 665                 670

atg cag cgc tgg gcc cgg ggc ccc aac ctc aca gcc act gtg gtc tgg   2122
Met Gln Arg Trp Ala Arg Gly Pro Asn Leu Thr Ala Thr Val Val Trp
    675                 680                 685

att gac ccc acc tat gtt gtg gcc aca tcc tat gac atc acg gta gat   2170
Ile Asp Pro Thr Tyr Val Val Ala Thr Ser Tyr Asp Ile Thr Val Asp
690                 695                 700                 705

gcg gac act gaa gtc acg cag tac aag ccc cca ctg agc ctg cca ctg   2218
Ala Asp Thr Glu Val Thr Gln Tyr Lys Pro Pro Leu Ser Leu Pro Leu
                710                 715                 720

cgg cca gga gcc tgg act gtt cga ttg ctt cag ttc tgg gag ccc ctg   2266
Arg Pro Gly Ala Trp Thr Val Arg Leu Leu Gln Phe Trp Glu Pro Leu
            725                 730                 735

ggt gag acc cgc ttc ctc gtg ctg cca ttg acc ttc aac cac aaa cta   2314
Gly Glu Thr Arg Phe Leu Val Leu Pro Leu Thr Phe Asn His Lys Leu
        740                 745                 750
```

```
cct ctc agg aaa gat gat gcc agc tgg ctg cat gcg gga cca ccc cac   2362
Pro Leu Arg Lys Asp Asp Ala Ser Trp Leu His Ala Gly Pro Pro His
    755                 760                 765

aac gaa tac atg gaa cag agt ttc cag gga cta agt ggc atc ctg aat   2410
Asn Glu Tyr Met Glu Gln Ser Phe Gln Gly Leu Ser Gly Ile Leu Asn
770                 775                 780                 785

ctg cct cag cca gag gcc gtg gag gag gct gcc cgg cgg cac aca gag   2458
Leu Pro Gln Pro Glu Ala Val Glu Glu Ala Ala Arg Arg His Thr Glu
                790                 795                 800

ctc aca ggt cct gca ctt gag gcc tgg aca gat ggg gaa ctg agc agt   2506
Leu Thr Gly Pro Ala Leu Glu Ala Trp Thr Asp Gly Glu Leu Ser Ser
            805                 810                 815

ttc tgg tct gtt gca gga ttg tgt gcc ata ggg cct tct tct tgt ccc   2554
Phe Trp Ser Val Ala Gly Leu Cys Ala Ile Gly Pro Ser Ser Cys Pro
        820                 825                 830

tcc ctg gag ctc tgc aga ctg acc agc tgg agc tct ctg tct cct gac   2602
Ser Leu Glu Leu Cys Arg Leu Thr Ser Trp Ser Ser Leu Ser Pro Asp
    835                 840                 845

ccc aag tca gag ctg ggg cct gtc aaa gct gac gga cga ctc agg tag   2650
Pro Lys Ser Glu Leu Gly Pro Val Lys Ala Asp Gly Arg Leu Arg
850                 855                 860                 865

caggacccca gccagcacaa cccggaggag ccggggaatt gcaccttaca gacaatggag 2710
ggacgtctct cctctggtag ccagggagcc ctagaacagt ggtggggctg tctgtctgtc 2770
aggctttcct aggaaatgga cgacatcagg ccctgctgag atccatgaag tttccccact 2830
ggaggaggga gccccactgg cagcacag                                    2858


<210> 8
<211> 864
<212> PRT
<213> Rat coronavirus

<400> 8
Met Val Ala Ser Ala Arg Val Gln Lys Leu Val Arg Arg Tyr Lys Leu
  1               5                  10                  15

Ala Ile Ala Thr Ala Leu Ala Ile Leu Leu Leu Gln Gly Leu Val Val
            20                  25                  30

Trp Ser Phe Ser Gly Leu Glu Glu Asp Glu Pro Gly Glu Lys Gly Arg
        35                  40                  45

Gln Arg Lys Pro Arg Pro Leu Asp Pro Gly Glu Gly Ser Lys Asp Thr
    50                  55                  60

Asp Ser Ser Ala Gly Arg Arg Gly Ser Ala Gly Arg Arg His Gly Arg
65                  70                  75                  80

Trp Arg Gly Arg Ala Glu Ser Pro Gly Val Pro Val Ala Lys Val Val
                85                  90                  95

Arg Ala Val Thr Ser Arg Gln Arg Ala Ser Arg Arg Val Pro Pro Ala
            100                 105                 110

Pro Pro Pro Glu Ala Pro Gly Arg Gln Asn Leu Ser Gly Ala Ala Ala
        115                 120                 125
```

```
Gly Glu Ala Leu Ile Gly Ala Ala Gly Phe Pro Gln His Gly Asp Thr
    130                 135                 140

Gly Ser Val Glu Gly Ala Pro Gln Pro Thr Asp Asn Ser Phe Thr Pro
145                 150                 155                 160

Lys Cys Glu Ile Val Gly Lys Asp Ala Leu Ser Ala Leu Ala Arg Ala
                165                 170                 175

Ser Thr Lys His Cys Gln Gln Glu Ile Ala Asn Val Val Cys Leu His
            180                 185                 190

Gln Ala Gly Asn Leu Met Pro Lys Ser Val Pro Arg His Cys Gln Leu
        195                 200                 205

Ala Gly Lys Val Ser Pro Gly Ile Gln Trp Glu Glu Val Arg Ala Gln
    210                 215                 220

Gln Pro Val Ser Gly Pro Leu Val Arg Ile Ala Tyr Met Leu Val Val
225                 230                 235                 240

His Gly Arg Ala Val Arg Gln Leu Lys Arg Leu Leu Lys Ala Val Tyr
                245                 250                 255

His Glu Glu His Phe Phe Tyr Ile His Val Asp Lys Arg Ser Asn Tyr
            260                 265                 270

Leu Tyr Arg Glu Val Val Glu Leu Ala Gln His Tyr Asp Asn Val Arg
        275                 280                 285

Val Thr Pro Trp Arg Met Val Thr Ile Trp Gly Gly Ala Ser Leu Leu
    290                 295                 300

Arg Met Tyr Leu Arg Ser Met Lys Asp Leu Leu Glu Thr Pro Gly Trp
305                 310                 315                 320

Thr Trp Asp Phe Phe Ile Asn Leu Ser Ala Thr Asp Tyr Pro Thr Arg
                325                 330                 335

Thr Asn Glu Glu Leu Val Ala Phe Leu Ser Lys Asn Arg Asp Lys Asn
            340                 345                 350

Phe Leu Lys Ser His Gly Arg Asp Asn Ser Arg Phe Ile Lys Lys Gln
        355                 360                 365

Gly Leu Asp Arg Leu Phe His Glu Cys Asp Ser His Met Trp Arg Leu
    370                 375                 380

Gly Glu Arg Gln Ile Pro Ala Gly Ile Val Val Asp Gly Gly Ser Asp
385                 390                 395                 400

Trp Phe Val Leu Thr Arg Ser Phe Val Glu Tyr Val Val Tyr Thr Glu
                405                 410                 415

Asp Pro Leu Val Ala Gln Leu Arg Gln Phe Tyr Thr Tyr Thr Leu Leu
            420                 425                 430

Pro Ala Glu Ser Phe Phe His Thr Val Leu Glu Asn Ser Pro Ala Cys
        435                 440                 445

Glu Ser Leu Val Asp Asn Asn Leu Arg Val Thr Asn Trp Asn Arg Lys
    450                 455                 460
```

```
Leu Gly Cys Lys Cys Gln Tyr Lys His Ile Val Asp Trp Cys Gly Cys
465                 470                 475                 480

Ser Pro Asn Asp Phe Lys Pro Gln Asp Phe Leu Arg Leu Gln Gln Val
                485                 490                 495

Ser Arg Pro Thr Phe Phe Ala Arg Lys Phe Glu Ser Thr Val Asn Gln
            500                 505                 510

Glu Val Leu Glu Ile Leu Asp Phe His Leu Tyr Gly Ser Tyr Pro Pro
        515                 520                 525

Gly Thr Pro Ala Leu Lys Ala Tyr Trp Glu Asn Ile Tyr Asp Met Ala
    530                 535                 540

Asp Gly Pro Ser Gly Leu Ser Asp Val Leu Leu Thr Ala Tyr Thr Ala
545                 550                 555                 560

Phe Ala Arg Ile Ser Leu Arg His Ala Ala Thr Val Ser Pro Leu Ala
                565                 570                 575

Thr Ala Val Cys Arg Phe Glu Pro Arg Gly Leu Pro Ser Ser Val His
            580                 585                 590

Leu Tyr Phe Tyr Asp Asp His Phe Gln Gly Tyr Leu Val Thr Gln Ala
        595                 600                 605

Val Gln Pro Ser Ala Gln Gly Pro Ala Glu Thr Leu Glu Met Trp Leu
    610                 615                 620

Met Pro Gln Arg Leu Leu Lys Pro Leu Gly His Ser Asp Gln Ala Ser
625                 630                 635                 640

Arg Leu Gln Ser Leu Glu Val Gly Thr Glu Trp Asp Pro Lys Glu Arg
                645                 650                 655

Leu Phe Arg Asn Phe Gly Gly Leu Leu Gly Pro Leu Asp Glu Pro Val
            660                 665                 670

Ala Met Gln Arg Trp Ala Arg Gly Pro Asn Leu Thr Ala Thr Val Val
        675                 680                 685

Trp Ile Asp Pro Thr Tyr Val Val Ala Thr Ser Tyr Asp Ile Thr Val
    690                 695                 700

Asp Ala Asp Thr Glu Val Thr Gln Tyr Lys Pro Pro Leu Ser Leu Pro
705                 710                 715                 720

Leu Arg Pro Gly Ala Trp Thr Val Arg Leu Leu Gln Phe Trp Glu Pro
                725                 730                 735

Leu Gly Glu Thr Arg Phe Leu Val Leu Pro Leu Thr Phe Asn His Lys
            740                 745                 750

Leu Pro Leu Arg Lys Asp Asp Ala Ser Trp Leu His Ala Gly Pro Pro
        755                 760                 765

His Asn Glu Tyr Met Glu Gln Ser Phe Gln Gly Leu Ser Gly Ile Leu
    770                 775                 780

Asn Leu Pro Gln Pro Glu Ala Val Glu Glu Ala Ala Arg Arg His Thr
785                 790                 795                 800
```

Glu Leu Thr Gly Pro Ala Leu Glu Ala Trp Thr Asp Gly Glu Leu Ser
805 810 815

Ser Phe Trp Ser Val Ala Gly Leu Cys Ala Ile Gly Pro Ser Ser Cys
820 825 830

Pro Ser Leu Glu Leu Cys Arg Leu Thr Ser Trp Ser Ser Leu Ser Pro
835 840 845

Asp Pro Lys Ser Glu Leu Gly Pro Val Lys Ala Asp Gly Arg Leu Arg
850 855 860

<210> 9
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: peptide

<400> 9
Cys Ser Arg Gln Lys Glu Leu Leu Lys Arg Lys Leu Glu Gln Gln Glu
1 5 10 15

Lys

<210> 10
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: peptide

<400> 10
Gln Glu Glu Glu Gly Ser Gly Gly Gly Gln Lys
1 5 10

<210> 11
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: peptide

<400> 11
Ser Glu Asn Glu Gly Ser Gly Met Ala Glu Gln Lys
1 5 10

<210> 12
<211> 12
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: peptide

<400> 12
Thr Glu Asn Glu Gly Ser Gly Leu Thr Asn Ile Lys
1 5 10

<210> 13
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 13
gtgggtatgc agaagtgggg gaagggac 28

<210> 14
<211> 18
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: primer

<400> 14
ccctccgcaa tgcctaca 18

<210> 15
<211> 20
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: primer

<400> 15
actgaggtca cgcaatacaa 20

<210> 16
<211> 25
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: primer

<400> 16
gccgcactca ggttgatgaa gaagt 25

<210> 17
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 17
accaccagga caaaggcgat tctga 25

<210> 18
<211> 20
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: primer

<400> 18
agtcgaacag tccagggccc 20

<210> 19
<211> 20
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: primer

<400> 19
cacgatctcg cacttggggg 20

<210> 20
<211> 32
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: primer

<400> 20
ctattcgatg atgaagatac cccaccaaac cc 32

<210> 21
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 21
ggaagagctg ggtgtggaat 20

<210> 22
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 22
tttcccgttg agatcctgct 20

<210> 23
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 23
acagacagca acaacgagaa 20

<210> 24
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 24
aaaggaaggc agaggaagc 19

<210> 25
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 25
acccctccac tgtctgtaag 20

<210> 26
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: digestion peptide

<400> 26
Glu Leu Gly Ala Lys
1 5

<210> 27
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: digestion peptide

<400> 27
Glu Leu Leu Lys
1

<210> 28
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: digestion peptide

<400> 28
Asp Met Asn Phe Leu Lys
1 5

<210> 29
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: digestion peptide

<400> 29
Ile Ala Ser Pro Pro Ser Asp Phe Gly Arg
1 5 10

<210> 30
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: digestion peptide

<400> 30
Leu Leu Leu Asp
1

<210> 31
<211> 13
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: digestion peptide

<400> 31
Asp Phe Glu Asn Val Asp Asn Ser Asn Phe Ala Pro Arg
1 5 10

<210> 32
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: digestion peptide

<400> 32
Pro Thr Phe Phe Ala Arg
1 5

<210> 33
<211> 12
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: digestion peptide

<400> 33
Leu Gln Phe Ser Glu Val Gly Thr Asp Xaa Asp Ala
1 5 10

<210> 34
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: digestion peptide

<400> 34
Glu Leu Gly Ala Val Lys Pro Asp Gly Arg Leu
1 5 10

<210> 35
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: digestion peptide

<400> 35
Glu Leu Leu Lys Arg Lys Leu Glu Gln Gln Glu Lys
1 5 10

<210> 36
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: digestion peptide

<400> 36
Leu Gly Leu Leu Met Pro Glu Lys
1 5